U.S. DEPARTMENT OF COMMERCE PATENT & TRADEMARK OFFICE

B/O Form PTO 1390 **Transmittal Letter to the United States Designated/Elected Office (DO/EO/US) Concerning a Filing Under 35 U.S.C. §371**		*Attorney's Docket Number* NITT.0049 10/009363
International Application Number **PCT/JP99/03209**	*International Filing Date* **June 16, 1999**	*Priority Date Claimed* **June 16, 1999**

Title of Invention
APPARATUS AND METHOD FOR GENE EXAMINATION

Applicant(s) for DO/EO/US
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1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. §371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. §371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. §371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. A copy of the International Application as filed (35 U.S.C. §371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. A English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. §371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☒ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. §371(c)(3)).
9. ☒ An unexecuted oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371(c)(5)).

Items 11 to 20 below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. §§1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§3.28 and 3.31 is included.
13. ☐ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C, 1.821-1.825
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. Other items or information:

Application Number (if known) 10/009363	*International Application Number* PCT/JP99/03209	*Attorney's Docket Number* NITT.0049

	CALCULATIONS	PTO USE ONLY
21. ☒ The following fees are submitted: **Basic National Fee** (37 C.F.R. §1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... 1,040.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... 890.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. ..... 740.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... 710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT	**$890.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. §1.492(e)).	**0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RAT		
Total Claims	43	23	x $18.	**$414.00**	
Independent Claims	20	17	x $84.	**$1428.00**	
Multiple Dependant Claims (if applicable)			+ 280.00		
TOTAL OF ABOVE CALCULATIONS				**2732.00**	
☐ Entity claims small entity status. See 37 CFR1.27. The fees indicated above are reduced by 1/2.				**0.00**	
SUBTOTAL				**2,732.00**	
Processing fee of **$130.00** for furnishing the English translation later than 20 30 months from the earliest claimed priority date (37 C.F.R. §1.492(f)).				**$0.00**	
TOTAL NATIONAL FEE				**2,732.00**	
Fee for recording the enclosed assignment (37 C.F.R. § 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§ 3.28, 3.31). **$40.00** per property				**$0.00**	
TOTAL FEES ENCLOSED				**$ 2, 732.00**	
				Amount to be Refunded:	**$**
				Charged:	**$**

a. ☒ A check in the amount of **$2,732.00** to cover the national fees is enclosed.

b. ☐ Please charge my **Deposit Account Number 08-1480** in the amount of **$**_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to **Deposit Account Number 08-1480**. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. **Provide credit card information and authorization on PTO-2038.**

Note.: Where an appropriate time limit under *37 C.F.R. §1.94 or* §1.*495* has not been met, a petition to revive *(37 C.F.R. §1.137(a) or (b))* must be filed and granted to restore the application to pending status.

Respectfully submitted,

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December 10, 2001

TITLE OF THE INVENTION

Apparatus and Method for Gene Examination

BACKGROUND OF THE INVENTION

This invention relates to an instrument for sample preparation for preparing samples in carrying out gene diagnosis using whole blood or a solution containing cultured cells, to an apparatus for gene examination with which gene examination, from sample preparation to detection of target genes, can be performed in a simple and easy manner, and to a method of gene examination.

The Human Genome Project was started in the 1980's on a worldwide scale and it is expected that information of the whole genome sequences will be obtained at the beginning of the 21st century. Once the whole genome sequence information is obtained, it will probably become possible to discriminate genes by gene types as to whether they are normal or abnormal and the development of gene examination methods and gene treatments or gene therapy using genetic engineering techniques will be accelerated sharply. The number of currently known gene-related diseases is said to amount to 8,450. In spite of this fact, the kinds of gene examination to which health insurance is applicable under the current system in Japan are limited to some of infectious diseases. This is because diseases resulting from gene mutation more

highly probably involve multiple mutations as compared with hereditary diseases due to monolithic mutation. Therefore, it is necessary to carry out the gene examination for a wide variety of genes, hence the examination becomes expensive; this is a problem from the medical management viewpoint. Another reason is that any appropriate examination method has not been established as yet, hence it is not yet possible to carry out the examination in a stable manner at any time and at any place.

The polymerase chain reaction (PCR) technique, which can serve as an effective technology for gene analysis for detecting target genes, is a technology capable of amplifying trace amounts of genes with high efficiency and can markedly improve the detection sensitivity.

A plurality of kits for extracting DNA or RNA from tissues or whole blood have been put on sale from Qiagen (Germany), Stratagene (USA) and other companies. The working time required for the extraction is about 30 minutes to about 3 hours and varies from kit to kit. Automated nucleic acid extractors have been put on sale from Toyobo, Kurabo and other companies. Also available as apparatus for real time detection of PCR products are ABIPRISM 7700 (Perkin Elmer, USA) and Light Cycler TM (Roche Diagnostics, Germany) among others.

In JP-A (Kokai) H04 (1992)-207195, there is

reported a method of collecting viral nucleotide fractions, or a method of testing for viruses, which comprises adding a resin capable of adsorbing viruses to a suspension containing a virus for capturing the virus, collecting the resin on a filter, passing a solution capable of lyzing the virus through the filter, recovering a viral nucleotide fraction-containing solution and thus collecting the nucleotide fraction for testing for viruses or for other purposes.

In JP-A H04 (1992)-36198, there is reported a method of testing for bacteria capable of causing food poisoning. According to the testing method described in JP-A H04-36198, a bacteria-containing suspension is passed through a first rough filter to separate an insoluble solid fraction. A lyzing reagent is then added to the filtrate to thereby cause bacterial components to be dissolved therein. A fine insoluble solid fraction is further removed by means of a second filter. To the thus-obtained filtrate is added an aggregation agent for nucleic acid components to precipitate nucleic acid components, and the nucleic acid components are captured in a third filter. This is washed, and an aqueous solution is passed through the third filter to elute the nucleic acid components. The thus-eluted nucleic acid components are subjected to a polymerase chain reaction to amplify the desired bacterial DNA and thereby detect the target organism causative of food poisoning.

In JP-A H07 (1995)-98314, there is reported an apparatus for testing for occult blood in stools. In the apparatus for testing for occult blood in stools as described in JP-A H07-98314, a solution prepared by dissolving a stool sample is first deprived of the solid matter by a separating filter. Then, the solid-free stool sample solution is dropped onto a measuring filter. When occult blood is present, blood hemoglobin (Hb) reacts, on the measuring filter, with a first antibody and a second antibody according to the sandwich method to form an immunocomplex. As a result, the measuring filter is colored. Conversely, if occult blood is absent, the measuring filter remains in an uncolored state. Therefore, the presence or absence of occult blood can readily be judged by judging, by the eye, as to whether the measuring filter has been colored or not colored.

SUMMARY OF THE INVENTION

Currently, there is no system existing for carrying out the steps of extraction of DNA or RNA from a sample, PCR and detection fully automatically. In manual operations, contamination with materials that affect the testing is highly possible between steps and thus there may arise the problem that test results may differ entirely from one another. The prior art apparatus and kits are all far from being fully automated. They perform a specific step or steps, for

example extraction alone or PCR amplification and PCR product detection alone, hence do not meet the requirements, from the sites of testing, that testing can be carried out anytime and anywhere in a simple and easy and stable manner. Namely, the problem of the existing kits or apparatus is that it is impossible to do all steps, from pretreatment to detection, by using only one application unit.

As explained hereinabove, when the currently available kits or apparatus are used, it is not possible to carry out all the steps, from pretreatment for extracting DNA or RNA to PCR amplification and PCR product detection, by using one and the same application unit but it is necessary to use several application units, and the problem is that the possibility of contamination by substances affecting the testing becomes very high between steps.

Accordingly, it is an object of the present invention to provide an apparatus for gene examination, a method of gene examination and an instrument for sample preparation, by which all the steps, from sample pretreatment step to PCR amplification and PCR product detection steps, can be carried out using one and the same application unit (sample preparation unit) and contamination between steps can be thereby prevented and which makes it easy to treat a number of specimens simultaneously.

In accordance with the invention, a sample

preparation instrument is used which has a plurality of sample preparation units (application units) each having a cell capturing filter and a polynucleotide capturing filter both equipped in advance. It is possible to continuously carry out the procedure comprising pouring whole blood or a cultured cell suspension, supplemented with a buffer solution for cells, into each sample preparation unit, then adding a denaturing agent, further adding a PCR amplification reaction mixture, amplifying a target gene and detecting the same. It is thus possible to carry out the steps from sample pretreatment step to PCR amplification and PCR product detection steps by using one and the same sample preparation unit. The contamination between steps can be avoided and a number of specimens can be easily treated simultaneously.

Typical constitutions of the invention have the following characteristics.

(C1) An apparatus for gene examination which comprises a sample preparation instrument comprising one or a plurality of sample preparation units each comprising an upper opening, into which a buffer solution with cells suspended therein is poured, a lower opening, through which a waste liquor is discharged, a channel connecting these openings and provided with a first filter for capturing the cells, a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured into

the upper opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore and a hydrophobic third filter, the three filters being arranged in the channel in that order in the direction from the upper opening to the lower opening, a member for holding the sample preparation unit or units and a means for controlling the temperature of the PCR amplification reaction mixture; an irradiation means for irradiating the PCR amplification reaction mixture with a laser beam capable of exciting the fluorophore label labeling the copy in the direction substantially perpendicular or substantially parallel to the second filter; and a detection means for detecting the fluorescence from the fluorophore label labeling the copy in the direction substantially perpendicular to the second filter.

(C2) A sample preparation unit which comprises an upper opening, into which a buffer solution with cells suspended therein is poured, a lower opening, through which a waste liquor is discharged, a channel connecting these openings and is provided with a first filter for capturing the cells, a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured into the upper opening and holding or retaining a PCR amplification

reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, and a hydrophobic third filter, the three filters being arranged in the channel in that order in the direction from the upper opening to the lower opening.

(C3) A method of gene examination using a sample preparation instrument comprising one or a plurality of sample preparation units each having a channel connecting an upper opening with a lower opening and a first filter, a second filter and a hydrophobic third filter arranged in the channel in that order in the direction from the upper opening to the lower opening, which method comprises (1) the step of pouring a buffer solution containing cells suspended therein into the upper opening of the sample preparation unit and capturing the cells on the first filter, (2) the step of pouring a denaturing agent into the upper opening and capturing polynucleotides thus eluted from the cells on the second filter, (3) the step of pouring, into the upper opening, a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore to thereby cause the PCR amplification reaction mixture to be held or

retained in the second filter and amplifying the copy and (4) the step of irradiating the PCR amplification reaction mixture with a laser beam capable of exciting the fluorophore label labeling the copy for detecting the fluorescence from the fluorophore label.

(C4) A sample preparation instrument which has a plurality of sample preparation units each comprising a first member having a first opening formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed in the lower part thereof for capturing the cells, a second member having a second opening formed at the top thereof and a second filter, in the lower part thereof, for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member having a hydrophobic third filter, and a means for controlling the temperature of the PCR amplification reaction mixture, the first member, the second member and the third member being arranged in that order from above.

(C5) A sample preparation instrument which has a plurality of sample preparation units each comprising a first member having a first opening formed at the top

thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed in the lower part thereof for capturing the cells, a second member having a second opening formed at the top thereof and a second filter, in the lower part thereof, for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member having a hydrophobic third filter, a transparent fourth member having a through hole, and a means for controlling the temperature of the PCR amplification reaction mixture, the first member, the fourth member, the second member and the third member being arranged in that order from above, the first filter and the second filter being opposed to each other via the through hole, and the second filter and the third filter being opposed to each other.

(C6) A sample preparation instrument which comprises a plurality of sample preparation units each comprising a first member having a first through hole, a first opening formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member having a second

opening at the top thereof and a second filter, in the lower part thereof, for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member having a hydrophobic third filter, and a means for controlling the temperature of the PCR amplification reaction mixture, the first member, the second member and the third member being arranged in that order from above, the first through hole and the second opening being opposed to each other, the second filter and the third filter being opposed to each other, and a channel being formed for connecting the first filter to the second filter.

(C7) A sample preparation instrument which comprises a plurality of sample preparation units each comprising a first member having a first through hole, a first opening formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member having a second filter, in the lower part thereof, for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and

holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member having a hydrophobic third filter and a transparent fourth member having a second through hole and a second opening at the top, and a means for controlling the temperature of the PCR amplification reaction mixture, the first member, the fourth member, the second member and the third member being arranged in that order from above, the first through hole and the second filter being opposed to each other via the second through hole, the second filter and the third filter being opposed to each other, and a channel being formed for connecting the first filter to the second filter.

(C8) An apparatus for gene examination which comprises a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member having a plurality of first regions each formed therein and having a first opening formed at the top, into which a buffer solution with cells suspended therein is poured, and a first filter formed in the lower part for capturing the cells, a second member having a plurality of second regions each formed therein and having a second opening at the top and a second filter for capturing polynucleotides

eluted from the cells by means of a denaturing agent poured into the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, a third member having a plurality of third regions each formed therein and having a hydrophobic third filter, and means for controlling the temperature of the PCR amplification reaction mixture, the first member, the second member and the third member being arranged in that order from above; an irradiation means for irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam capable of exciting the fluorophore label labeling the copy in the direction substantially perpendicular to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units; and a detection means for detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter.

(C9) An apparatus for gene examination which comprises a sample preparation instrument comprising a

plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member having a plurality of first regions each formed therein and having a first opening formed at the top, into which a buffer solution with cells suspended therein is poured, and a first filter formed in the lower part for capturing the cells, a second member having a plurality of second regions each formed therein and having a second opening at the top and a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured into the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, a third member having a plurality of third regions each formed therein and having a hydrophobic third filter, a transparent fourth member having a plurality of fourth regions each having a through hole formed therein, and means for controlling the temperature of the PCR amplification reaction mixture, the first member, the fourth member, the second member and the third member being arranged in that order from above, the first filter and the second filter being opposed to each other via the through hole, and the second filter and the third filter being opposed to each other; an irradiation

means for irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam capable of exciting the fluorophore label labeling the copy in the direction substantially parallel to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units; and a detection means for detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter.

(C10) An apparatus for gene examination which comprises a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member having a plurality of first regions each formed therein and having a first through hole, a first opening formed at the top, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member having a plurality of second regions each formed therein and having a second opening at the top and a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured into the first opening and holding or retaining a PCR amplification

reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, a third member having a plurality of third regions each formed therein and having a hydrophobic third filter, and means for controlling the temperature of the PCR amplification reaction mixture, the first member, the second member and the third member being arranged in that order from above, the first through hole and the second opening being opposed to each other, the second filter and the third filter being opposed to each other, and a channel being formed for connecting the first filter to the second filter; an irradiation means for irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam capable of exciting the fluorophore label labeling the copy in the direction substantially perpendicular to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units; and a detection means for detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter.

(C11) An apparatus for gene examination which comprises a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member having a plurality of first regions each formed therein and having a through hole, a first opening formed at the top, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member having a plurality of second regions each formed therein and having a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured into the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, a third member having a plurality of third regions each formed therein and having a hydrophobic third filter, a transparent fourth member having a plurality of fourth regions each formed therein and having a through hole and a second opening at the top, and means for controlling the temperature of the PCR amplification reaction mixture, the first member, the fourth member, the second member and the third member being arranged in that order from above, the first through hole and the second filter being

opposed to each other via the second through hole, the second filter and the third filter being opposed to each other, and a channel being formed for connecting the first filter to the second filter; an irradiation means for irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam capable of exciting the fluorophore label labeling the copy in the PCR reaction mixture in the direction substantially parallel to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units; and a detection means for detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter.

(C12) A method of gene examination using a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second and a third region, and thus comprising a first member having a plurality of first regions each formed therein and having a first opening formed at the top and a first filter formed in the lower part thereof, a second member having a plurality of second regions each formed therein and having a second opening at the top and a second filter in the lower part thereof and a third

member having a plurality of third regions each formed therein and having a hydrophobic third filter, with the first member, the second member and the third member being arranged in that order from above, which method comprises (1) the step of pouring a buffer solution with cells suspended therein into the first opening and capturing the cells on the first filter, (2) the step of pouring a denaturing agent into the first opening and capturing polynucleotides thereby eluted from the cells on the second filter, (3) pouring a PCT amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, into the first opening, allowing the PCR amplification reaction mixture to be held or retained by the second filter and amplifying the copy, (4) the step of irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam capable of exciting the fluorophore label labeling the copy in the direction substantially perpendicular to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units and (5) the step of detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction

mixture in each sample preparation unit in the direction substantially perpendicular to the second filter.

(C13) A method of gene examination using a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member having a plurality of first regions each formed therein and having a first opening formed at the top and a first filter formed in the lower part thereof, a second member having a plurality of second regions each formed therein and having a second opening at the top and a second filter in the lower part thereof, a third member having a plurality of third regions each formed therein and having a hydrophobic third filter and a transparent fourth member having a plurality of fourth regions each formed therein and having a through hole, with the first member, the fourth member, the second member and the third member being arranged in that order from above, the first filter and the second filter being opposed to each other via the through hole and the second filter and the third filter being opposed to each other, which method comprises (1) the step of pouring a buffer solution with cells suspended therein into the first opening and capturing the cells on the first filter, (2) the step of pouring a denaturing agent into the first opening and capturing polynucleotides thereby eluted from the cells on the

second filter, (3) pouring a PCT amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, into the first opening, allowing the PCR amplification reaction mixture to be held or retained and amplifying the copy, (4) the step of irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam capable of exciting the fluorophore label labeling the copy in the PCR amplification mixture in each sample preparation unit in the direction substantially parallel to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units and (5) the step of detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter in each sample preparation unit.

(C14) A method of gene examination using a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second and a third region, and thus comprising a first member having a plurality of first regions each formed therein and having a first through hole, a first opening formed at

the top and a first filter formed below the first opening, a second member having a plurality of second regions each formed therein and having a second opening at the top and a second filter in the lower part thereof and a third member having a plurality of third regions each formed therein and having a hydrophobic third filter, with the first member, the second member and the third member being arranged in that order from above, the first through hole and the second opening being opposed to each other, the second filter and the third filter being opposed to each other and a channel being formed for connecting the first filter to the second filter, which method comprises (1) the step of pouring a buffer solution with cells suspended therein into the first opening and capturing the cells on the first filter, (2) the step of pouring a denaturing agent into the first opening and capturing polynucleotides thereby eluted from the cells on the second filter, (3) pouring a PCT amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, into the first opening, allowing the PCR amplification reaction mixture to be held or retained and amplifying the copy, (4) the step of irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam capable of exciting

the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units and (5) the step of detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter in each sample preparation unit.

(C15) A method of gene examination using a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member having a plurality of first regions each formed therein and having a first through hole, a first opening formed at the top, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member having a plurality of second regions each formed therein and having a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured from the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR

amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member having a plurality of third regions each formed therein and having a hydrophobic third filter and a transparent fourth member having a plurality of fourth regions each formed therein and having a second through hole and a second opening at the top, with the first member, the fourth member the second member and the third member being arranged in that order from above, the first through hole and the second filter being opposed to each other via the second through hole, the second filter and the third filter being opposed to each other and a channel being formed for connecting the first filter to the second filter, which method comprises (1) the step of pouring a buffer solution with cells suspended therein into the first opening and capturing the cells on the first filter, (2) the step of pouring a denaturing agent into the first opening and capturing polynucleotides thereby eluted from the cells on the second filter, (3) pouring a PCT amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, into the first opening, allowing the PCR amplification reaction mixture to be held or retained and amplifying the copy, (4) the step

of irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam capable of exciting the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially parallel to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units and (5) the step of detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter.

(C16) A sample preparation instrument which has a plurality of sample preparation units, each having a first, a second and a third region, and thus comprising a first member having a plurality of first regions each formed therein and having a first opening formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed in the lower part thereof for capturing the cells, a second member having a plurality of second regions each formed therein and having a second opening formed at the top thereof and a second filter, in the lower part thereof, for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR

amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member having a plurality of third regions each formed therein and having a hydrophobic third filter, and means for controlling the temperature of the PCR amplification reaction mixture, the first member, the second member and the third member being arranged in that order from above.

(C17) A sample preparation instrument which comprises a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member having a plurality of first regions each formed therein and having a first opening formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed in the lower part thereof for capturing the cells, a second member having a plurality of second regions each formed therein and having a second opening at the top thereof and a second filter, in the lower part thereof, for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with

a fluorophore, a third member having a plurality of third regions each formed therein and having a hydrophobic third filter, a transparent fourth member having a plurality of fourth regions each formed therein and having a through hole, and means for controlling the temperature of the PCR amplification reaction mixture, the first member, the fourth member, the second member and the third member being arranged in that order from above, the first filter and the second filter being opposed to each other via the through hole, and the second filter and the third filter being opposed to each other.

(C18) A sample preparation instrument which comprises a plurality of sample preparation units, each having a first, a second and a third region, and thus comprising a first member having a plurality of first regions each formed therein and having a first through hole, a first opening formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member having a plurality of second regions each formed therein and having a second opening at the top and a second filter, in the lower part thereof, for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction

for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member having a plurality of third regions each formed therein and having a hydrophobic third filter, and means for controlling the temperature of the PCR amplification reaction mixture, the first member, the second member and the third member being arranged in that order from above, the first through hole and the second opening being opposed to each other, the second filter and the third filter being opposed to each other, and a channel being formed for connecting the first filter to the second filter.

(C19) A sample preparation instrument which comprises a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member having a plurality of first regions each formed therein and having a first through hole, a first opening formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member having a plurality of second regions each formed therein and having a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction

for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member having a plurality of third regions each formed therein and having a hydrophobic third filter, a transparent fourth member having a plurality of fourth regions each formed therein and having a second through hole and a second opening at the top, and means for controlling the temperature of the PCR amplification reaction mixture, the first member, the fourth member, the second member and the third member being arranged in that order from above, the first through hole and the second filter being opposed to each other via the second through hole, the second filter and the third filter being opposed to each other, and a channel being formed for connecting the first filter to the second filter.

(C20) A sample preparation instrument which comprises a sample preparation unit having an upper opening, into which a buffer solution with cells suspended therein is poured, a lower opening for discharging a waste liquor, a channel connecting the openings, a first filter for capturing the cells, a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial

base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, and a hydrophobic third filter, the filters being arranged in that order in the channel in the direction from the upper opening to the lower opening; a holding member for holding the sample preparation unit; and a means for controlling the temperature of the PCR amplification reaction mixture.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a perspective view showing a constitution example of the sample preparation instrument in a first embodiment of the invention.

Fig. 2 is a sectional view showing the section along A-A′ in Fig. 1.

Fig. 3 is a sectional view illustrating a process for purifying a whole blood sample using the sample preparation instrument according to the first embodiment of the invention.

Fig. 4 is an illustration showing the principle of fluorescence resonance energy transfer which is utilized in the first embodiment of the invention.

Fig. 5 is a sectional view showing a constitution example of a gene detection apparatus for real-time detection of the process of amplification of a target gene using an optical fiber in the first embodiment of the invention.

Fig. 6 is a sectional view showing a

constitution example of the gene detection apparatus shown in Fig. 5 in which an optical fiber covered by a sheath having an edge-like tip is used.

Fig. 7 is a sectional view showing a constitution example of the gene detection apparatus for real-time detection of the process of target gene amplification under irradiation of a sample preparation unit with a laser beam from below in the first embodiment of the invention.

Fig. 8 is a sectional view showing a constitution example of the gene detection apparatus for real-time detection of the process of target gene amplification under irradiation of a sample preparation unit with a laser beam in the horizontal direction in the first embodiment of the invention.

Fig. 9 is a perspective view showing a constitution example of the sample preparation instrument having a plurality of sample preparation units in a second embodiment of the invention.

Fig. 10 is a sectional view showing a constitution example of a gene detection apparatus for real-time detection of the process of target gene amplification in the second embodiment of the invention.

Fig. 11 is a sectional view showing a constitution example of a gene detection apparatus for real-time detection of the process of target gene amplification under irradiation of a plurality of sample preparation units with a laser beam in the

horizontal direction in the second embodiment of the invention.

Fig. 12 is a sectional view showing a constitution example of a laser irradiation and fluorescence detection system for two-dimensional scanning in a gene diagnosis apparatus in the second embodiment of the invention.

Fig. 13 is a sectional view showing a constitution example of a laser irradiation and fluorescence detection system for one-dimensional scanning in a gene diagnosis apparatus in the second embodiment of the invention.

Fig. 14 is a perspective view showing a constitution example of the sample preparation set in a third embodiment of the invention.

Fig. 15 is an illustration showing a method of purifying a plurality of whole blood samples and a process for detecting a target gene amplification product in a fourth embodiment of the invention in which a cassette type sample preparation instrument is used.

Fig. 16 is a perspective view showing a constitution example of the sample preparation instrument having sample preparation units for simultaneously treating a plurality of samples in a fifth embodiment of the invention.

Fig. 17 is a sectional view showing the section along A-A' in Fig. 16.

Fig. 18 is an illustration showing the structure of each layer constituting the sample preparation instrument in the fifth embodiment of the invention.

Fig. 19 is a perspective view showing a constitution example of the sample preparation instrument for simultaneously treating a plurality of samples in a sixth embodiment of the invention.

Fig. 20 is a sectional view showing the section along A-A' in Fig. 19.

Fig. 21 is an illustration showing the structure of each layer constituting the sample preparation instrument in the sixth embodiment of the invention.

Fig. 22 is a perspective view showing a modified constitution example of the sample preparation instrument of the sixth embodiment of the invention.

Fig. 23 is a perspective view showing a constitution example of the sample preparation instrument for simultaneously treating a plurality of sample in a seventh embodiment of the invention.

Fig. 24 is a sectional view showing the section along A-A' in Fig. 23.

Fig. 25 is an illustration showing the structure of each layer constituting the sample preparation instrument in the seventh embodiment of the invention.

Fig. 26 is a plan view showing a constitution

example of the sample preparation instrument for simultaneously treating a plurality of samples in an eighth embodiment of the invention.

Fig. 27 is a sectional view showing a constitution example of the sample preparation instrument in the eighth embodiment of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the following, several embodiments are described in detail referring to the drawings.

EMBODIMENT I

<Pretreatment of blood samples>

Whole blood is collected from each of patients with lung cancer or subjects having a checkup for cancer. About one milliliter (mL) is sufficient as the blood sample size. The whole blood collected is subjected to anticoagulation treatment with sodium citrate.

<Purification of total RNA from the sample blood>

Fig. 1 is a perspective view showing a constitution example of the sample preparation instrument according to an embodiment of the invention and Fig. 2 is a sectional view showing the section along A-A' in Fig. 1. As shown in Fig. 1 and Fig. 2, the sample preparation instrument 50 is constituted of a sample preparation unit 100, a holding member 200 for holding the sample preparation unit 100 and a heating block 210. The sample preparation unit 100 has an axis

of rotational symmetry and is constituted of an upper hollow cylindrical part having a large first diameter, a lower hollow cylindrical part having a small second diameter and a bottle neck portion. The upper hollow cylindrical part and bottle neck portion of the sample preparation unit are put in a hollow portion formed in the holding member 200. The lower hollow cylindrical part of the sample preparation unit is placed in a hollow portion formed in the heating block 210. In the sample preparation unit shown in Fig. 1, a filter holder 120 for holding a first filter 110, a second filter 130 and a filter holder 150 for holding a third filter 140 have been omitted.

The sample preparation unit 100 has an upper opening for charging a buffer solution with cells suspended therein, a lower opening for discharging a waste liquor and a channel connecting these and further comprises a first filter (cell capturing filter) 110 for capturing target cells, for example leukocytes, a second filter (polynucleotide capturing filter) 130 for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the upper opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, and a hydrophobic third filter (liquid holding filter

for holding the PCR amplification reaction mixture) 140, the three filters being arranged in the channel in that order in the direction from the upper opening to the lower opening.

For capturing target cells such as leukocytes, the first filter has a thickness of 0.05 mm to 0.5 mm and a pore size of 1 ìm to 3 ìm. For capturing the polynucleotides eluted from the cells and holding the PCR amplification reaction mixture, the second filter has a thickness of 0.1 mm to 0.5 mm and a pore size of 10 ìm to 40 ìm. For holding the PCR amplification reaction mixture, the third filter has a thickness of 0.1 mm to 0.5 mm and a pore size of 10 ìm to 40 ìm. The first filter 110 is held by the filter holder 120, and the second filter 130 and third filter 140 are supported by the filter holder 150 made of a plastic material or the like. The filter holders 120 and 140 maintain a constant flow of each liquid charged into the sample preparation unit from above and prevent the first, second and third filters from being damaged upon exertion of pressure on the first, second and third filters.

The first filter 110 and filter holder 120 are disposed in the upper hollow cylindrical part of the sample preparation unit, and the second filter 130, third filter 140 and filter holder 150 are disposed in the lower hollow cylindrical part of the sample preparation unit. In the heating block 210, there is

embedded a Peltier device for raising and lowering the temperature of the solution retained in the second filter 130 disposed in the lower hollow cylindrical part of the sample preparation unit and in a space above the filter 130.

As for the size of the sample preparation instrument 50 shown in Fig. 1 and Fig. 2, the lengths in the x, y and z directions are, for example, 25 mm, 25 mm and 60 mm, respectively. The holding member 200 and heating block 210 are, for example, 50 mm and 10 mm long in the z direction, respectively. The outside diameter and inside diameter of the upper hollow cylindrical part of the sample preparation unit 100 are, for example, 20 mm and 18 mm, respectively, and the outside diameter and inside diameter of the lower hollow cylindrical part are, for example 10 mm and 8 mm, respectively.

Fig. 3 is a sectional view showing the process for purifying a whole blood specimen using the sample preparation instrument according to the embodiment of the invention. In Fig. 3, the holding member 200 for holding the sample preparation unit 100, the third filter 140 and the heating block 210 (except for step 6) have been omitted for simplicity's sake. For disrupting erythrocytes in the whole blood collected under osmotic pressure, 9 mL of a 50 mM NaCl solution is added to each mL of the blood specimen and the whole is stirred. Immediately after stirring, 10 mL of the

solution 300 containing disrupted erythrocytes is poured into a space above the first filter 110 of the sample preparation unit 100 (step 1).

Under the action of gravity, the solution 300 passes through the first filter 110 and flows down into a space below the first filter 110. However, a majority of the solution 300 hardly passes through the first filter 110 under the action of gravity alone. Therefore, pressure is exerted from above the sample preparation unit 100 to cause the solution 300 to pass through the first filter 110. On that occasion, leukocytes 302 alone in the solution 300 are captured by the first filter 110. The solution that has passed through the first filter 110 passes through the space below the first filter 110, the second filter 130 and the third filter 140 and flows out into a waste liquor tank. In lieu of the procedure for applying pressure, a procedure for applying centrifugal force or a procedure for suction from the lower opening, for instance, may be utilized for causing the solution to pass through the first, second and third filters. Then, 3 mL of a denaturing agent 310 is poured into a space above the first filter 110 (step 2).

Like in step 1, pressure is exerted from above the sample preparation unit 100 to thereby cause the denaturing agent 310 to pass through the first filter 110. On that occasion, the membrane of the leukocytes 302 captured on the first filter 110 is disrupted and

components 304 other than RNA 320 remain on the first filter 110, and the RNA 320 eluted from the leukocytes 302 passes through the space below the first filter 110 and is captured on the second filter 130. The denaturing agent 310 passes through the third filter 140 and flows out into the waste liquor tank. The second filter 130 preferably has a sufficient thickness to hold the RNA to a satisfactory extent. For example, the second filter 130 has a thickness of 1 mm. In the above manner, the total RNA 320 alone, out of the whole blood collected, can be captured on the second filter 130 (step 3).

<Amplification of a target gene from the total RNA>

Using the total RNA purified from the whole blood collected, PCR amplification of the target gene is carried out for diagnostic purposes. According to the presence or absence of this gene amplification product or the extent of amplification of the product, the subject can be diagnosed as to the occurrence or nonoccurrence of cancer metastasis or as to the advancement of cancer, for instance. A PCR amplification reaction mixture comprising a buffer solution for PCR, two primers for amplifying the target gene and four deoxynucleotides is prepared in advance. A 50-ìL portion of the PCR amplification reaction mixture 340 is poured into a space above the first filter 110 (step 4).

Then, pressure is exerted from above the sample

preparation unit 100. On that occasion, the pressure to be applied is adjusted to a rather low level so that the PCR amplification reaction mixture 340 can pass the first filter 110 but cannot pass the second filter 130. By adjusting the pressure to be applied, the PCR amplification reaction mixture 340 is retained in a state such that the second filter 130 is soaked or filled therewith and the excess PCR amplification reaction mixture that the second filter 130 cannot retain is retained in a space below the first filter but above the second filter 130 in the form of a mixture 330 thereof with the total RNA 320. The solution 330 containing the total RNA 320 will not leak out of the sample preparation unit owing to the presence of the hydrophobic third filter 140 (step 5).

During the PCR, the raising and lowering of the temperature of the mixture retained in the second filter 130 and in the space above the second filter 130 is controlled by means of the heating block 210. The PCR amplification of the target gene is effected in the solution 330 retained in the second filter 130 and in the space above the third filter 140 to give a solution containing the PCR amplification product 335 (step 6).

<Real-time detection of the PCR product>

According to a first method for real-time detection of the PCR amplification product, a phosphor capable of preferentially binding to the newly synthesized (amplified) double-stranded DNA and being

markedly enhanced in fluorescence intensity is admixed with the PCR amplification reaction mixture 340 in advance. As the phosphor, SYBR Green 1 (product of Molecular Probe), for instance, is used. SYBR Green 1 binds to the newly synthesized (amplified) double-stranded DNA, so that the fluorescence intensity is directly proportional to the DNA concentration, hence the PCR amplification product can be detected quantitatively. By detecting the fluorescence intensity while carrying out the PCR amplification reaction, it is possible to monitor the process of amplification of the PCR product.

A second method for real-time detection of the PCR amplification product utilizes the principle of fluorescence resonance energy transfer (FRET).

Fig. 4 illustrates the principle of fluorescence resonance energy transfer to be utilized in this example of the invention. FRET occurs only when two probes hybridize with the amplification product in close vicinity to each other. A hybridization probe 23 labeled with a donor dye (F1) 22 at the 3' terminus anneals with a single-stranded template DNA 21 resulting from denaturation and a hybridization probe 25 labeled with an acceptor dye (F2) 24 at the 5' terminus hybridizes with the template at a site adjacent to the hybridization probe 23. The donor dye (F1) 22 is excited by light 26 from a light emitting diode (LED) and the donor dye (F1) 22 emits

light having an excitation wavelength for the acceptor dye (F2) 24. The acceptor dye (F2) 24 emits light 27 with a longer wavelength by secondary excitation.

In cases where any specific DNA allowing such adjacent hybridization of the hybridization probes 23 and 25 therewith is not present, the phenomenon of FRET will not occur. As the amount of the PCR product increases, the amount of the probes hybridizing with the single-stranded template DNA resulting from denaturation increases. The intensity of the light 27 resulting from FRET is proportional to the PCR product formed from the specific DNA with which the hybridization probes 23 and 25 can hybridize adjacently to each other. By detecting the light 27 due to FRET, it is thus possible to monitor the process of amplification of the PCR product on the real time basis.

Fig. 5 is a sectional view showing a constitution example of the gene detection apparatus to be used in the first embodiment of the invention for real-time detection of the process of amplification of a target gene using an optical fiber. The optical fiber 31 is inserted into the sample preparation unit 100 from above, the fiber 31 is passed through the first filter 110 by breaking the filter by applying pressure to the central portion thereof, and the optical fiber 31 is allowed to advance to a position close to the level of the solution containing the PCR amplification product 335 and retained on the second

filter 130. Then, a laser beam 380 emitted from a light source 370 is reflected by a dichroic mirror 385 to irradiate the solution containing the PCR amplification product 335 via the fiber 31. Upon irradiation with the laser beam 380, fluorescent light is emitted from the fluorophore label in the PCR amplification product, and the fluorescent light passes through the dichroic mirror 385 and is detected by a CCD camera 400.

Fig. 6 is a sectional view showing a constitution example of the gene detection apparatus in which the optical fiber in the constitution example shown in Fig. 5 is covered with a sheath having an edge-like tip. The optical fiber 31 having an outside diameter of 100 ìm is placed in a sheath 42 having an outside diameter of 140 ìm and an inside diameter of 120 ìm, and the end of the sheath 42 is cut by a plane crossing with the axis of the hollow cylinder to form an edge-like tip 41. The constitution example shown in Fig. 6 is the same as the constitution shown in Fig. 5 except that the optical fiber 31 is covered with the sheath 42 having an edge-like tip 41. A hole is made in the central portion of the first filter 110 by breaking the filter by means of the sheath 42 and the sheath 42 is caused to advance to a level above the surface of the solution containing the PCR amplification product 335. In this condition, the optical fiber 31 is pushed from the inside of the

sheath 42 to come out of the sheath 42, and the fiber 31 is caused to approach close to the surface of the solution containing the PCR amplification product 335. The fluorescence emitted upon irradiation with a laser beam is detected in the same manner as in the case shown in Fig. 5.

The process of amplification of the target gene can also be detected on the real time basis without using any optical fiber.

Fig. 7 is a sectional view showing a constitution example of the gene detection apparatus for real-time detection of the process of target gene amplification in the first embodiment of the invention by irradiating a laser beam from below the sample preparation unit. The laser beam 380 enters the sample preparation unit 100 from below and passes through the third filter 140, the solution containing the PCR amplification product 335 as retained by the second filter 130 is irradiated with the transmitted laser beam 380, and the fluorescence 395 from the fluorophore label in the PCR amplification product can be detected on the real time basis by a photomultiplier tube 390 disposed below the lower opening of the sample preparation unit.

Fig. 8 is a sectional view showing a constitution example of the gene detection apparatus for real-time detection of the process of target gene amplification in the first embodiment of the invention,

in which apparatus the sample preparation unit is irradiated with a laser beam in the horizontal direction. In the constitution example shown in Fig. 8, the holding member 200″ holding the sample preparation unit 100 is made of a transparent material, and the solution containing the PCR amplification product 335 as retained by the second filter 130 is irradiated with the laser beam 380. In cases where the holding member 200″ is made of an opaque material, a hole may be provided for the passage of the laser beam 380.

In the same manner as the case shown in Fig. 7, the fluorescence 395 from the fluorophore label in the PCR amplification product can be detected on the real time basis by the photomultiplier tube 390 disposed below the lower opening of the sample preparation unit. That portion of the sample preparation unit 100 which is irradiated with the laser beam is made of a transparent material.

Referring to Fig. 7 and Fig. 8, it is desirable that the second filter 130 and the third filter 140 be transparent. Even if the second filter 130 and/or third filter 140 is not transparent, however, the laser beam 380 that has passed through pores of the third filter 140 can pass through pores of the second filter 130 and, thereby, the solution containing the PCR amplification product 335 is irradiated therewith. The fluorescence 395 emitted by the fluorophore label in the PCR amplification product passes through pores of

the second filter 130 and through pores of the third filter 140 and arrives at the photomultiplier tube 390, so that the fluorescence can be readily detected on the real time basis.

In place of the real time detection of the PCR product, the product may be detected after completion of the PCR amplification. Thus, pressure is applied from above the sample preparation unit, and the solution after completion of the PCR amplification is recovered in a recovery receptacle disposed at the lower opening of the sample preparation unit. The recovery receptacle is then irradiated with a laser beam and the fluorescence from the fluorophore label in the PCR amplification product is detected. The PCR amplification product can thus be quantitated.

As explained above, by real time quantitative analysis of the process of PCR amplification of a target gene suited for diagnosis or by quantitation of the amplification product after completion of the PCR amplification reaction, it is possible to make a diagnosis as to the occurrence or nonoccurrence of metastasis of lung cancer, for instance, or the extent of advancement of metastasis, etc.

EMBODIMENT II

Fig. 9 is a perspective view showing a constitution example of the sample preparation instrument having a plurality of sample preparation units according to a second embodiment of the invention.

The sample preparation instrument 50' shown in Fig. 9 has a constitution such that a plurality of the same sample preparation units 50 as shown in Fig. 1 and Fig. 2 (in Fig. 9, the case of 9 units is shown for simplicity's sake) are integrated in two crossing directions. The sample preparation instrument 50' is constituted of a holding member 200' for holding a plurality of sample preparation units 100, and a heating block 210'. The upper hollow cylindrical part and bottle neck portion of each sample preparation unit 100 are contained in each hollow space formed in the holding member 200'. The lower hollow cylindrical part of each sample preparation unit is contained in each hollow space formed in the heating block 210'. With 10 sample preparation units 50 as shown in Fig. 1 and Fig. 2 in the x direction and 10 such units in the y direction, the sample preparation instrument 50' has a size of 250 mm x 250 mm.

In the sample preparation instrument 50' shown in Fig. 9, the treatment steps to be carried out with one specimen as explained referring to the first embodiment, namely purification of total RNA from a specimen, target gene amplification from the total RNA and real-time detection of the PCR product, can all be carried out simultaneously with a plurality of specimens.

As for the total RNA purification from blood specimens, the procedure for purification of total RNA

from each blood specimen is carried out independently in each sample preparation unit 100 in the same manner as in the first embodiment but in parallel and simultaneously. The PCR amplification of a target gene from the total RNA is carried out in the solution retained by the second filter 130 and third filter 140 in each sample preparation unit 100 in the same manner as in the first embodiment.

Fig. 10 is a sectional view showing a constitution example of the gene detection apparatus for real-time detection of the process of amplification of the target gene. Using the same constitution as shown in Fig. 5 or Fig. 6, each optical fiber 91 is brought into contact, from above each sample preparation unit, with the solution containing the PCR amplification product 335 retained by the second filter 130. The solution containing the PCR amplification product 335 is irradiated with a laser beam 380 from a light source 370. The laser beam 380 is sent to each optical fiber 91 by means of a beam splitter comprising an array of dichroic mirrors 385. The fluorescence emitted from each solution containing the PCR amplification product 335 is collected, via the dichroic mirror 385, by a condenser lens 96 and detected by a CCD camera 400.In Fig. 10, the three sample preparation units placed side by side in the y direction in each of the three lines shown in Fig. 9 are irradiated with the laser beam 380 simultaneously

and the fluorescence from each of the sample preparation units in each y direction line is detected on the real time basis simultaneously for the three units.

Fig. 11 is a perspective view showing a constitution example of the gene detection apparatus for real-time detection of the process of target gene amplification in the second embodiment of the invention in which apparatus a plurality of sample preparation units of the sample preparation instrument 50″ are irradiated with a laser beam in the horizontal direction. Like the constitution example shown in Fig. 8, the holding member 200″ for holding the sample preparation units 100 is made of a transparent material. In cases where the holding member 200″ is made of an opaque material, holes may be provided for the passage of the laser beam 380. Those portions of the sample preparation units 100 which are to be irradiated with a laser beam are made of a transparent material.

The laser beam 380 is reflected by dichroic mirrors 385 for simultaneous irradiation of the three sample preparation units 100 placed side by side in each of the three lines in the direction of the axis y and thus the solution containing the PCR amplification product 335 as retained by the second filter 130 of each of the three sample preparation units 100 is irradiated with the laser beam. In each sample preparation unit 100, the fluorescence 395 from the

fluorophore label in the PCR amplification product is detected on the real time basis by a CCD camera 400 disposed below the lower opening of the sample preparation instrument 50". The fluorescence 395 may also be detected from the upper openings of the sample preparation instrument 50" by disposing the CCD camera 400 accordingly.

Referring to Fig. 11, it is desirable that the second filter 130 and third filter 140 in each sample preparation unit 100 be transparent. Even if the second filter 130 and/or third filter 140 is not transparent, however, the laser beam 380 that has passed through pores of the third filter 140 can pass through pores of the second filter 130 and, thereby, the solution containing the PCR amplification product 335 is irradiated therewith. The fluorescence 395 emitted by the fluorophore label in the PCR amplification product passes through pores of the second filter 130 and through pores of the third filter 140 and arrives at a photomultiplier tube 390, so that the fluorescence can be readily detected on the real time basis.

In the sample preparation instrument 50' shown in Fig. 11, a plurality of sample preparation units can be laser-irradiated, so that the processes of PCR amplification of a target gene suited for diagnostic purposes in the plurality of sample preparation units can be analyzed simultaneously on the real time basis.

In lieu of the laser irradiation system comprising the light source 370 and the CCD camera 400 for fluorescence detection as shown in Fig. 11, the laser irradiation and fluorescence detection system shown in Fig. 12 may be used for two-dimensional scanning in the x and y directions, or the laser irradiation and fluorescence detection system shown in Fig. 13 for one-dimensional scanning in the x or y direction, so that each sample preparation unit may be irradiated with a laser beam from above and the fluorescence may be detected through the upper opening of each sample preparation unit. It is a matter of course that the plurality of photomultiplier tubes 390 may be replaced with a CCD camera 400.

As explained above, by real time quantitative analysis of the processes of PCR amplification of a target gene suited for diagnosis for a plurality of samples, it is possible to make a diagnosis as to the occurrence or nonoccurrence of metastasis of lung cancer, for instance, or the extent of advancement of metastasis, etc., for the plurality of samples.

EMBODIMENT III

It is also possible to use such a sample preparation unit 100' as shown in Fig. 14 in lieu of the sample preparation unit 100 explained referring to the first and second embodiments. The sample preparation unit 100' has an axis of rotational symmetry and is constituted of an upper hollow prism-

like part having a square section with longer sides, a lower hollow prism-like part having a square section with shorter sides and a neck portion connecting the upper hollow prism-like part with the lower hollow prism-like part. The upper hollow prism-like part and the neck portion of the sample preparation unit are contained in a hollow space formed in a holding member. The lower hollow prism-like part of the sample preparation unit is placed in a hollow space formed in a heating block. The height of the sample preparation unit 100' is the same as that of the sample preparation unit 100 and the sectional area of the upper hollow prism-like part and that of the lower hollow prism-like part are, for example, 16 mm x 16 mm and 5 mm x 5 mm, respectively.

EMBODIMENT IV

The pretreatment of blood specimens is performed following the same procedure as in the first embodiment.

<Purification of total RNA from blood specimens>

Fig. 15 is an illustration showing the method of purifying a plurality of whole blood specimens and the step of detecting the target gene amplification product using a cassette-type sample preparation instrument in a fourth embodiment of the invention. To 1 mL of each blood sample collected is added 9 mL of a 50 mM NaCl solution, and the whole is stirred. Immediately after stirring, each sample solution is

poured into each independent well of a first cassette 800 having a plurality of wells each formed therein and equipped, at the bottom, with a first filter 110 and a filter holder 120 for holding the first filter. Separate specimens are prepared for respective separate wells. A gas pressure is applied from above the first cassette 800, or suction is made from below the first cassette 800, to thereby cause leukocytes alone in each solution to be captured on the first filter.

Then, a second cassette 810 having wells corresponding to the wells in the first cassette 800 and equipped, at the bottom of each well, with a second filter, a third filter and a filter holder 150 (not shown) for holding the second and third filters is attached to the bottom of the first cassette 800. Then, a denaturing agent is poured into each well of the first cassette 800. A gas pressure is applied from above the first cassette 800, or suction is made from below the second cassette 810, to thereby cause the denaturing agent to pass through the first and second filters, and the total RNA alone as derived from each whole blood sample collected is captured each independently on the second filter 130 of each well of the second cassette 810.

<Target gene amplification from the total RNA>

Using each purified total RNA fraction, amplification of a target gene for diagnosis is carried out. A third cassette 820 for PCR and amplification

product detection is attached to the bottom of the second cassette 810 and a PCR amplification reaction mixture is poured into each well of the second cassette 810 and, then, a gas pressure is applied from above the second cassette 810, or suction is made from below the third cassette 820, and the RNA captured by each second filter 130 of the second cassette 810 is eluted into the PCR amplification reaction mixture when this reaction mixture passes through the second filter 130. The RNA is thus transferred to each corresponding well of the third cassette 820. Then, the third cassette 820 alone is set on a heating block 210' (not shown in Fig. 15), and the PCR of the target gene is conducted.

<PCR product detection>

After completion of the PCR, each well of the third cassette 820 is irradiated with a laser beam using the laser irradiation and fluorescence detection system shown in Fig. 12 or Fig. 13, and the target gene amplification product is detected and quantitated for each well of the third cassette independently.

EMBODIMENT V

Referring to a fifth embodiment of the invention, Fig. 16 is a perspective view showing an constitution example of the sample preparation instrument having sample preparation units for simultaneous treatment of a plurality of specimens, Fig. 17 is a sectional view showing the section along A-A' in Fig. 16, and Fig. 18 is an illustration showing the

structure of each layer constituting the sample preparation instrument of the fifth embodiment. It goes without saying that the sample preparation instrument may have, in lieu of the constitution shown in Figs. 16 to 18, a constitution such that there is only one sample preparation unit.

In the fifth embodiment, the sample preparation instrument 550-1 is constituted of a first substrate 500, a second substrate 520, a third substrate 530 and a fourth substrate 540. In the sample preparation instrument 550-1, a plurality of sample preparation units are integrated two-dimensionally and different specimens are prepared in different sample preparation units.

The first substrate 500 is provided with a plurality of upper openings 511-1, 512-1, 513-1 and 514-1, into which separate solutions containing disrupted red blood cells (erythrocytes) (each solution can be obtained from each sample in the same manner as in the first embodiment) are poured respectively. A first filter is formed at the bottom of each opening. The second substrate 520 has upper openings 511-2, 512-2, 513-2 and 514-2 opposed to the upper openings 511-1, 512-1, 513-1 and 514-1, respectively, and a second filter is formed at the bottom of each opening. The third substrate 530 is provided, at the top, with third filters opposed to the respective second filters each formed at the bottom of each of the upper openings 511-

2, 512-2, 513-2 and 514-2 and, below the respective third filters, lower openings 511-3, 512-3, 513-3 and 514-3 are formed. The fourth substrate 540 has through holes 511-4, 512-4, 513-4 and 514-4 formed opposed to the lower openings 511-3, 512-3, 513-3 and 513-4, respectively. In the fourth substrate 540, there are embedded Peltier devices for temperature controlling in raising and lowering the temperature of the solution retained in each second filter and in the space above the second filter for effecting the PCR amplification.

The first to fourth substrates can be obtained by liquid-phase etching or dry etching of silicon. For example, the first substrate has a thickness of 500 ìm, the first filter has a thickness of 1 ìm to 10 ìm, the first filter has a plurality of square pores with a sectional area of (1 ìm to 3 ìm) x (1 ìm to 3 ìm), the second substrate has a thickness of 500 ìm, the second filter has a thickness of 10 ìm to 100 ìm, the second filter has a plurality of square pores with a sectional area of about 10 ìm x about 10 ìm, the third substrate has a thickness of 500 ìm, the third filter has a thickness of 10 ìm to 100 ìm, and the third filter is surface-treated to acquire hydrophobicity and has a plurality of square pores with a sectional area of (2 ìm to 3 ìm) x (2 ìm to 3 ìm). The openings in the first, second and third substrates have bottom areas of 500 ìm x 500 ìm, 500 ìm x 500 ìm and 500 ìm x 500 ìm, respectively and the through holes in the fourth

substrate have a sectional area of 500 ìm x 500 ìm. The first, second, third and fourth substrates are piled up one by one and finally integrated. When it has 20 sample preparation units in each of the x and y directions, the sample preparation instrument 550-1 has a size of 20 mm x 20 mm.

The total RNA purification from blood samples and the target gene amplification from the total RNA are carried out according to the same procedure as in the first embodiment. The PCR amplification product-containing solution 571, 572, 573 or 574 retained in each second filter and in the space above the second filter is irradiated with a laser beam 380, as shown in Fig. 19, and the fluorescence emitted from the PCR amplification product-containing solution is detected in the direction of irradiation with the laser beam, by two-dimensional scanning in the x and y directions using the laser irradiation and fluorescence detection system shown in Fig. 12 or by one-dimensional scanning in the x or y direction using the laser irradiation and fluorescence detection system shown in Fig. 13. It is also possible to irradiate the PCR amplification product-containing solution retained in each second filter and in the space above the second filter with a laser beam 380 from each opening in the first substrate and detect the fluorescence from each opening in the fourth substrate. Conversely, the solution may be irradiated with a laser beam 380 from each opening in

the fourth substrate and the fluorescence detected from each opening in the first substrate. Further, like the constitution shown in Fig. 5, Fig. 6 or Fig. 10, the first substrate may be perforated so that an optical fiber can be inserted to a position close to the PCR amplification product-containing solution retained in the space above the second filter to detect the fluorescence emitted upon laser irradiation on the real time basis.

EMBODIMENT VI

Referring to a sixth embodiment of the invention, Fig. 19 is a perspective view showing a constitution example of the sample preparation instrument for treating a plurality of samples simultaneously, Fig. 20 is a sectional view showing the section along A-A' in Fig. 19, and Fig. 21 is an illustration showing the structure of each layer constituting the sample preparation instrument according to the sixth embodiment. It goes without saying that the sample preparation instrument may have, in lieu of the constitution shown in Figs. 19 to 21, a constitution such that there is only one sample preparation unit.

In the sample preparation instrument 550-2, like in the fifth embodiment, a plurality of sample preparation units are integrated two-dimensionally and different specimens are prepared in different sample preparation units. In the constitution of the sample

preparation instrument 550-2 according to the sixth embodiment, the constitution of the sample preparation instrument 550-1 of the fifth embodiment is supplemented with a transparent fifth substrate 510 disposed between the first substrate 500 and the second substrate 520. The fifth substrate 510 has through holes 511-5, 512-5, 513-5 and 514-5 respectively opposed to the first filters in the first substrate 500 and to the upper openings 511-2, 512-2, 153-2 and 514-2 in the second substrate. The fifth substrate 510 has a thickness of 500 ìm and each through hole has a sectional area of 500 ìm x 500 ìm.

The total RNA purification from blood samples and the target gene amplification from the total RNA are carried out according to the same procedure as in the first embodiment. The PCR amplification product-containing solution 571, 572, 573 or 574 retained in the space above each second filter is irradiated with a laser beam 380 from one lateral side of the fifth substrate 510 (in the y direction), as shown in Fig. 20, using the laser irradiation system shown in Fig. 11, and the fluorescence 395 is detected by a CCD camera 400 from below the sample preparation instrument 550-2 through the third and fourth openings, like in the constitution shown in Fig. 11. It is also possible to detect the fluorescence 395 by the CCD camera 400 from above the sample preparation instrument 550-2 through the first openings.

Fig. 22 is a perspective view showing a modified constitution example of the sample preparation instrument according to the sixth embodiment of the present invention. In the constitution of the sample preparation instrument 550-3 shown in Fig. 22, a plurality of sample preparation units are integrated one-dimensionally, and different specimens are prepared in different sample preparation units. As shown in Fig. 22, each PCR amplification product-containing solutions 571, 572, 573 or 574 retained in the space above each second filter is irradiated with a laser beam 380 from a lateral side of the fifth substrate 510 (in the x direction) and the fluorescence 395 is detected by the CCD camera 400 from another lateral side of the fifth substrate 510 (in the y direction) in a direction intersecting the direction of laser irradiation. It is also possible to detect the fluorescence 395 from above or from below the sample preparation instrument 550-3. It goes without saying that the sample preparation instrument may have, in lieu of the constitution shown in Fig. 22, a constitution such that there is only one sample preparation unit.

EMBODIMENT VII

Referring to a seventh embodiment of the invention, Fig. 23 is a perspective view showing a constitution example of the sample preparation instrument for treating a plurality of samples simultaneously, Fig. 24 is a sectional view showing the

section along A-A' in Fig. 23, and Fig. 25 is an illustration showing the structure of each layer constituting the sample preparation instrument according to the seventh embodiment. It goes without saying that the sample preparation instrument may have, in lieu of the constitution shown in Figs. 23 to 25, a constitution such that there is only one sample preparation unit.

In the seventh embodiment, the sample preparation instrument 650-1 is constituted of a first substrate 600, a second substrate 620, a third substrate 630 and a fourth substrate 640. In the sample preparation instrument 650-1, a plurality of sample preparation units are integrated two-dimensionally, and different specimens are prepared in different sample preparation units.

The first substrate 600 is provided with a plurality of upper openings 611-1, 612-1, 613-1 and 614-1, into which separate solutions containing disrupted erythrocytes (each solution can be obtained from each sample in the same manner as in the first embodiment) are poured respectively. A first filter is formed at the bottom of each opening. The first substrate 600 is provided with through holes 601, 602, 603 and 604 close to the openings 611-1, 612-1, 613-1 and 614-1, respectively. In the second substrate 620, there are formed upper openings 611-2, 612-2, 613-2 and 614-2 opposed respectively to the upper openings 611-1,

612-1, 613-1 and 614-1 of the first substrate and second upper openings 611'-2, 612'-2, 613'-2 and 614'-2 each having a second filter at the bottom and respectively opposed to the through holes 601, 602, 603 and 604 of the first substrate and connected with the first upper openings 611-2, 612-2, 613-2 and 614-2. The second upper opening is deeper than the first upper opening. In the third substrate 630, there are formed third filters opposed to the respective second filters each formed at the bottom of each of the upper openings 611'-2, 612'-2, 613'-2 and 614'-2 and, below the respective third filters, lower openings 611-3, 612-3, 613-3 and 614-3 are formed. The fourth substrate 640 has through holes 611-4, 612-4, 613-4 and 614-4 formed opposed to the lower openings 611-3, 612-3, 613-3 and 613-4, respectively. In the fourth substrate 640, there are embedded Peltier devices for temperature controlling in raising and lowering the temperature of the solution retained in each second filter and in the space above the second filter for effecting the PCR amplification.

The first to fourth substrates can be obtained by liquid-phase etching or dry etching of silicon. For example, the first substrate has a thickness of 500 ìm, the first filter has a thickness of 1 ìm to 10 ìm, the first filter has a plurality of square pores with a sectional area of (1 ìm to 3 ìm) x (1 ìm to 3 ìm), the second substrate has a thickness of 500 ìm, the second

filter has a thickness of 10 ìm to 100 ìm, the second filter has a plurality of square pores with a sectional area of about 10 ìm x about 10 ìm, the third substrate has a thickness of 500 ìm, the third filter has a thickness of 10 ìm to 100 ìm, and the third filter is surface-treated to acquire hydrophobicity and has a plurality of square pores with a sectional area of (2 ìm to 3 ìm) x (2 ìm to 3 ìm). The openings in the first substrate each has a bottom area of 500 ìm x 500 ìm, and the through holes in the first substrate each has a sectional area of 500 ìm x 500 ìm. When it has 20 sample preparation units in each of the x and y directions, the sample preparation instrument 650-1 has a size of 20 mm x 20 mm.

The total RNA purification from blood samples and the target gene amplification from the total RNA are carried out according to the same procedure as in the first embodiment. The PCR amplification product-containing solution 571, 572, 573 or 574 retained in the space above each second filter is irradiated with a laser beam through the through hole 601, 602, 603 or 604 in the first substrate, and the fluorescence emitted from the PCR amplification product-containing solution is detected, using the laser irradiation and photodetector system shown in Fig. 12 or Fig. 13. It is also possible to irradiate the PCR amplification product-containing solutions with a laser beam through the through holes 601, 602, 603 and 604 using the laser

irradiation system shown in Fig. 11 and detect the fluorescence from each solution by a CCD camera 400 through each through hole in the fourth substrate.

The sample preparation instrument 650-1 according to the seventh embodiment is characterized in that the first filter and the second filter are not opposed to each other, that the upper opening of the second filter is small in bottom area and great in depth, hence the PCR amplification product-containing solution can be contained in a minute volume and that since the PCR amplification product-containing solution is directly irradiated with a laser beam for detecting the fluorescence, the fluorescence can be directly detected and, therefore, the processes of PCR amplification of a target gene for diagnostic purposes can simultaneously be analyzed quantitatively with high sensitivity.

EMBODIMENT VIII

Referring to an eighth embodiment of the invention, Fig. 26 is a plan view showing a constitution example of the sample preparation instrument for treating a plurality of samples simultaneously, and Fig. 27 is a sectional view showing a constitution example of the sample preparation instrument according to the eighth embodiment. It goes without saying that the sample preparation instrument may have, in lieu of the constitution shown in Figs. 26 and 27, a constitution such that there is only one

sample preparation unit.

In the sample preparation instrument 650-2 according to the eighth embodiment, the constitution of the sample preparation instrument 650-1 in the seventh embodiment is modified in a manner such that the second substrate 620 is constituted of a transparent substrate 620a and a substrate 620b. In the substrate 620a, there are formed first upper openings 611-2, 612-2, 613-2 and 614-2 opposed respectively to the upper openings 611-1, 612-1, 613-1 and 614-1 of the first substrate and through holes 611'-2, 612'-2, 613'-2 and 614'-2 respectively opposed to the through holes 601, 602, 603 and 604 of the first substrate and connected with the first upper openings 611-2, 612-2, 613-2 and 614-2. In the substrate 620b, there are formed second filters opposed to the respective through holes 611'-2, 612'-2, 613'-2 and 614'-2 of the substrate 620a. By integrating the substrate 620a and substrate 620b, there are formed second upper openings 611'-2, 612'-2, 613'-2 ad 614'-2 above the second filters of the substrate 620b.

As shown in Fig. 26 and Fig. 27, the PCR amplification product-containing solution 571, 572, 573 or 574 retained in the space above the second filter in each sample preparation unit of the sample preparation instrument is irradiated with a laser beam 380 from a lateral side of the substrate 620a and the fluorescence is detected by a CCD camera 400 through the through

hole in the first substrate. It is also possible to detect the fluorescence by a CCD camera 400 through the through hole in the fourth substrate.

The sample preparation instrument according to the eighth embodiment is characterized in that a plurality of sample preparation units can be irradiated simultaneously with a laser beam, that the fluorescence from each PCR amplification product-containing solution can be detected directly and that the processes of PCR amplification of a target gene suited for diagnosis in a plurality of sample preparation units can be simultaneously analyzed quantitatively with high sensitivity.

In the fifth to eighth embodiments explained hereinabove, the substrates can be produced from silicon substrates by the photolithography technology and etching technology. Therefore, the pore sizes of first, second and third filters, the sectional areas of through holes, the depths of openings and the bottom area of openings, among others, on silicon substrates can be controlled with very good precision, so that sample preparation instruments having a plurality of minute sample preparation units uniform in performance characteristics can be provided at low cost. Further, since p-n junctions for detecting the heater resistance and temperature can be integrally formed in silicon substrates by diffusion layer formation, the temperature cycle in the PCR amplification reaction can

be performed with silicon chips alone without using any external heater temperature controlling, hence small-sized sample preparation instruments can be produced. Furthermore, since silicon is small in heat capacity, the temperature cycle in the PCR amplification reaction can be repeated at a high speed.

An area of 1 mm x 1 mm, for instance, is sufficient for one sample preparation unit to occupy, and a sample preparation instrument having about 300 sample preparation units can be produced using a silicon substrate having a size of 1 inch x 1 inch. Thus, it becomes possible to perform gene examination using a very small sample amount, for example about 0.5 iL.

In the above description, the case of extraction, amplification and detection of RNA derived from leukocytes in whole blood has been taken as an example. It goes without saying that the test samples or specimens to which the present invention is applicable include not only leukocytes in whole blood but also RNA species extracted from various organ- or tissue-derived cells included in whole blood, RNA species extracted from cells cultured in a cell culture fluid, and so on.

In the sample preparation instrument according to the invention, a series of operations from pouring a buffer solution with whole blood suspended therein, a buffer solution with cells cultured in a cell culture

medium suspended therein, a buffer solution with tissue cells suspended therein or the like into a sample application opening of the sample preparation instrument having a first, a second and a third filter, then adding a cell-disrupting reagent, a denaturing agent for eluting RNA from cells and a PCR amplification reaction mixture successively to thereby purify total RNA and amplify a target gene from the total RNA, to detecting the target gene is carried out continuously, so that contamination between operations can be prevented and a number of specimens can be easily treated simultaneously.

CLAIMS

1. An apparatus for gene examination which comprises a sample preparation instrument (50, 50', 50") comprising one or a plurality of sample preparation units (100, 100') each comprising an upper opening, into which a buffer solution with cells suspended therein is poured, a lower opening, through which a waste liquor is discharged, a channel connecting these openings and provided with a first filter (110) for capturing the cells, a second filter (130) for capturing polynucleotides eluted from the cells by means of a denaturing agent poured into the upper opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore and a hydrophobic third filter (140), the three filters being arranged in the channel in that order in the direction from the upper opening to the lower opening, a holding member (200, 200') for holding the sample preparation unit or units and a means (210, 210') for controlling the temperature of the PCR amplification reaction mixture; an irradiation means (370, 380, 385, 31) for irradiating the PCR amplification reaction mixture with a laser beam (380) capable of exciting the fluorophore label labeling the copy in the direction substantially

perpendicular or substantially parallel to the second filter; and a detection means (385, 390, 400) for detecting the fluorescence (395) from the fluorophore label labeling the copy in the direction substantially perpendicular to the second filter.

2. An apparatus for gene examination as claimed in Claim 1, wherein the sample preparation instrument has a plurality of sample preparation units as defined in Claim 1 disposed along a straight line, the irradiation means irradiates the PCR amplification reaction mixtures in the sample preparation units substantially simultaneously with the laser beam along the straight line in the direction substantially parallel to the second filter in each sample preparation unit, the detection means detects the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixtures in the sample preparation units substantially simultaneously in the direction substantially perpendicular to the second filter in each sample preparation unit and the sites of the holding means which are irradiated with the laser beam are constituted of a material (200″) transparent to the wavelength of the laser beam and to the wavelength of the fluorescence.

3. An apparatus for gene examination as claimed in Claim 1, wherein the sample preparation instrument has a plurality of lines of a plurality of sample preparation units as defined in Claim 1 disposed

along a straight line, the irradiation means irradiates the PCR amplification reaction mixtures in the sample preparation units in each of the lines substantially simultaneously with the laser beam along each straight line in the direction substantially parallel to the second filter in each sample preparation unit, the detection means detects the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixtures in each line of the sample preparation units substantially simultaneously in the direction substantially perpendicular to the second filter in each sample preparation unit in each line and the sites of the holding means which are irradiated with the laser beam are constituted of a material (200") transparent to the wavelength of the laser beam and to the wavelength of the fluorescence.

4. An apparatus for gene examination as claimed in Claim 1, wherein the sample preparation instrument has a plurality of sample preparation units as defined in Claim 1 disposed along a straight line, the irradiation means irradiates the PCR amplification reaction mixtures in the sample preparation units substantially simultaneously with the laser beam in the direction substantially perpendicular to the second filter in each sample preparation unit and the detection means detects the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixtures in the sample

preparation units substantially simultaneously in the direction substantially perpendicular to the second filter in each sample preparation unit.

5. An apparatus for gene examination as claimed in Claim 1, wherein the sample preparation instrument has a plurality of lines of a plurality of sample preparation units as defined in Claim 1 disposed along a straight line, the irradiation means irradiates the PCR amplification reaction mixtures in the sample preparation units in each of the lines substantially simultaneously with the laser beam in the direction substantially perpendicular to the second filter in each sample preparation unit in each line and the detection means detects the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixtures in each line of the sample preparation units substantially simultaneously in the direction substantially perpendicular to the second filter in each sample preparation unit in each line.

6. A sample preparation unit which comprises an upper opening, into which a buffer solution with cells suspended therein is poured, a lower opening, through which a waste liquor is discharged, a channel connecting these openings and is provided with a first filter (110) for capturing the cells, a second filter (130) for capturing polynucleotides eluted from the cells by means of a

denaturing agent poured into the upper opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, and a hydrophobic third filter (140), the three filters being arranged in the channel in that order in the direction from the upper opening to the lower opening.

7. A sample preparation unit as claimed in Claim 6, wherein there is a space for holding or retaining the PCR amplification reaction mixture between the first filter and the second filter.

8. A sample preparation unit as claimed in Claim 6, wherein the second filter has an area smaller than the area of the first filter.

9. A sample preparation unit as claimed in Claim 6, wherein the second filter is in contact with the third filter.

10. A sample preparation unit as claimed in Claim 6 which is constituted of a transparent material.

11. A sample preparation unit as claimed in Claim 6 which has an axis of rotational symmetry.

12. A sample preparation unit as claimed in Claim 11 the external form of which has a circular or square section perpendicular to the axis of rotational symmetry thereof.

13. A method of gene examination using a

sample preparation instrument (50, 50', 50") comprising one or a plurality of sample preparation units (100, 100') each having a channel connecting an upper opening with a lower opening and a first filter (110), a second filter (130) and a hydrophobic third filter (140) arranged in the channel in that order in the direction from the upper opening to the lower opening, which method comprises (1) the step of pouring a buffer solution containing cells suspended therein into the upper opening of the sample preparation unit and capturing the cells on the first filter, (2) the step of pouring a denaturing agent into the upper opening and capturing polynucleotides thus eluted from the cells on the second filter, (3) the step of pouring, into the upper opening, a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore to thereby cause the PCR amplification reaction mixture to be held or retained in the second filter and amplifying the copy and (4) the step of irradiating the PCR amplification reaction mixture with a laser beam capable of exciting the fluorophore label labeling the copy for detecting the fluorescence from the fluorophore label.

14. A method of gene examination as claimed in Claim 13, wherein the PCR amplification reaction mixture is irradiated with the laser beam in the

direction substantially perpendicular or substantially parallel to the second filter and the fluorescence is detected in the direction substantially perpendicular to the second filter.

15. A method of gene examination as claimed in Claim 13, wherein the sample preparation instrument has a plurality of sample preparation units as defined in Claim 13 disposed along a straight line, those sites of the holding member which are irradiated with the laser beam are constituted of a material (200") transparent to the wavelength of the laser beam and to the wavelength of the fluorescence, the PCR amplification reaction mixtures in the sample preparation units are irradiated substantially simultaneously with the laser beam along the straight line in the direction substantially parallel to the second filter in each sample preparation unit and the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixtures in the sample preparation units is detected substantially simultaneously in the direction substantially perpendicular to the second filter in each sample preparation unit.

16. A method of gene examination as claimed in Claim 13, wherein the sample preparation instrument has a plurality of lines of a plurality of sample preparation units as defined in Claim 13 disposed along a straight line, those sites of the holding member

which are irradiated with the laser beam are constituted of a material (200″) transparent to the wavelength of the laser beam and to the wavelength of the fluorescence, the PCR amplification reaction mixtures in the sample preparation units are irradiated substantially simultaneously with the laser beam along the straight line in each line in the direction substantially parallel to the second filter in each sample preparation unit and the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixtures in the sample preparation units in each line is detected substantially simultaneously in the direction substantially parallel to the second filter in each sample preparation unit in each line.

17. A method of gene examination as claimed in Claim 13, wherein the sample preparation instrument has a plurality of sample preparation units as defined in Claim 13 disposed along a straight line, the PCR amplification reaction mixtures in the sample preparation units are irradiated substantially simultaneously with the laser beam in the direction substantially perpendicular to the second filter in each sample preparation unit and the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixtures in the sample preparation units is detected substantially simultaneously in the direction substantially

perpendicular to the second filter in each sample preparation unit.

18. A method of gene examination as claimed in Claim 13, wherein the sample preparation instrument has a plurality of lines of a plurality of sample preparation units as defined in Claim 13 disposed along a straight line, the PCR amplification reaction mixtures in the sample preparation units in each line are irradiated substantially simultaneously with the laser beam in the direction substantially perpendicular to the second filter in each sample preparation unit and the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixtures in the sample preparation units in each line is detected substantially simultaneously in the direction substantially perpendicular to the second filter in each sample preparation unit in each line.

19. A sample preparation instrument which has a plurality of sample preparation units each comprising a first member (500, 600) having a first opening (511-1, 512-1, 513-1, 514-4; 611-1, 612-1, 613-1, 614-1) formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed in the lower part thereof for capturing the cells, a second member (520, 620) having a second opening (511-2, 512-2, 513-2, 514-2; 611-2, 612-2, 613-2, 614-2; 611'-2, 612'-2, 613'-2, 614'-2) formed at the top thereof and a second filter, in the

lower part thereof, for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member (530, 630) having a hydrophobic third filter, and a means (540, 640) for controlling the temperature of the PCR amplification reaction mixture, the first member, the second member and the third member being arranged in that order from above.

20. A sample preparation instrument as claimed in Claim 19, wherein the first member, second member and third member are each constituted of a silicon substrate and the pores of the first filer, second filter and third filter are pores formed in the respective silicon substrates.

21. A sample preparation instrument as claimed in Claim 19, wherein the second filter has an area smaller than the area of the first filter.

22. A sample preparation instrument which has a plurality of sample preparation units each comprising a first member (500) having a first opening (511-1, 512-1, 513-1, 514-1) formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed in the

lower part thereof for capturing the cells, a second member (520) having a second opening (511-2, 512-2, 513-2, 514-2) formed at the top thereof and a second filter, in the lower part thereof, for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member (530) having a hydrophobic third filter, a transparent fourth member (510) having a through hole (511-5, 512-5, 513-5, 514-5), and a means (540) for controlling the temperature of the PCR amplification reaction mixture, the first member, the fourth member, the second member and the third member being arranged in that order from above, the first filter and the second filter being opposed to each other via the through hole, and the second filter and the third filter being opposed to each other.

23. A sample preparation instrument as claimed in Claim 22, wherein the first member, second member and third member are each constituted of a silicon substrate and the pores of the first filer, second filter and third filter are pores formed in the respective silicon substrates.

24. A sample preparation instrument as

claimed in Claim 22, wherein the second filter has an area smaller than the area of the first filter.

25. A sample preparation instrument which comprises a plurality of sample preparation units each comprising a first member (600) having a first through hole (601, 602, 603, 604), a first opening (611-1, 612-1, 613-1, 614-1) formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member (620) having a second opening (611-2, 612-2, 613-2, 614-2; 611'-2, 612'-2, 613'-2, 614'-2) at the top thereof and a second filter, in the lower part thereof, for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member (630) having a hydrophobic third filter, and a means (640) for controlling the temperature of the PCR amplification reaction mixture, the first member, the second member and the third member being arranged in that order from above, the first through hole and the second opening (611'-2, 612'-2, 613'-2, 614'-2) being opposed to each other, the second filter and the third filter being opposed to each other, and a

channel being formed for connecting the first filter to the second filter.

26. A sample preparation instrument as claimed in Claim 25, wherein the first member, second member and third member are each constituted of a silicon substrate and the pores of the first filer, second filter and third filter are pores formed in the respective silicon substrates.

27. A sample preparation instrument as claimed in Claim 25, wherein the second filter has an area smaller than the area of the first filter.

28. A sample preparation instrument which comprises a plurality of sample preparation units each comprising a first member (600) having a first through hole (601, 602, 603, 604), a first opening (611-1, 612-1, 613-1, 614-1) formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member (620b) having a second filter, in the lower part thereof, for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member (630) having a hydrophobic third filter

and a transparent fourth member (620a) having a second through hole (611'-2, 612'-2, 613'-2, 614'-2) and a second opening (611-2, 612-2, 613-2, 614-2) at the top, and a means (640) for controlling the temperature of the PCR amplification reaction mixture, the first member, the fourth member, the second member and the third member being arranged in that order from above, the first through hole and the second filter being opposed to each other via the second through hole, the second filter and the third filter being opposed to each other, and a channel being formed for connecting the first filter to the second filter.

29. A sample preparation instrument as claimed in Claim 28, wherein the first member, second member and third member are each constituted of a silicon substrate and the pores of the first filer, second filter and third filter are pores formed in the respective silicon substrates.

30. A sample preparation instrument as claimed in Claim 28, wherein the second filter has an area smaller than the area of the first filter.

31. An apparatus for gene examination which comprises a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member (500, 600) having a plurality of first regions each formed therein and having a first opening (511-1, 512-1, 513-1, 514-1; 611-1, 612-1, 613-

1, 614-1) formed at the top, into which a buffer solution with cells suspended therein is poured, and a first filter formed in the lower part for capturing the cells, a second member (520, 620) having a plurality of second regions each formed therein and having a second opening (511-2, 512-2, 513-2, 514-2; 611-2, 612-2, 613-2, 614-2; 611'-2, 612'-2, 613'-2, 614'-2) at the top and a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured into the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, a third member (530, 630) having a plurality of third regions each formed therein and having a hydrophobic third filter, and means (540, 640) for controlling the temperature of the PCR amplification reaction mixture, the first member, the second member and the third member being arranged in that order from above; an irradiation means for irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam 380 capable of exciting the fluorophore label labeling the copy in the direction substantially perpendicular to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units; and a detection means for

detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter.

32. An apparatus for gene examination which comprises a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member (500) having a plurality of first regions each formed therein and having a first opening (511-1, 512-1, 513-1, 514-1) formed at the top, into which a buffer solution with cells suspended therein is poured, and a first filter formed in the lower part for capturing the cells, a second member (520) having a plurality of second regions each formed therein and having a second opening (511-2, 512-2, 513-2, 514-2) at the top and a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured into the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, a third member (530) having a plurality of third regions each formed therein and having a hydrophobic third

filter, a transparent fourth member (510) having a plurality of fourth regions each having a through hole (511-5, 512-5, 513-5, 514-5) formed therein, and a means (540) for controlling the temperature of the PCR amplification reaction mixture, the first member, the fourth member, the second member and the third member being arranged in that order from above, the first filter and the second filter being opposed to each other via the through hole, and the second filter and the third filter being opposed to each other; an irradiation means for irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam capable of exciting the fluorophore label labeling the copy in the direction substantially parallel to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units; and a detection means for detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter.

33. An apparatus for gene examination which comprises a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member (600) having a plurality of

first regions each formed therein and having a first through hole (601, 602, 603, 604), a first opening (611-1, 612-1, 613-1, 614-1) formed at the top, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member (620) having a plurality of second regions each formed therein and having a second opening (611-2, 612-2, 613-2, 614-2; 611'-2, 612'-2, 613'-2, 614'-2) at the top and a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured into the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, a third member (630) having a plurality of third regions each formed therein and having a hydrophobic third filter, and a means (640) for controlling the temperature of the PCR amplification reaction mixture, the first member, the second member and the third member being arranged in that order from above, the first through hole and the second opening (611'-2, 612'-2, 613'-2, 614'-2) being opposed to each other, the second filter and the third filter being opposed to each other, and a channel being formed for connecting the first filter to the second filter; an irradiation means for irradiating the PCR

amplification reaction mixture in each sample preparation unit with a laser beam (380) capable of exciting the fluorophore label labeling the copy in the direction substantially perpendicular to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units; and a detection means for detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter.

34. An apparatus for gene examination which comprises a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member (600) having a plurality of first regions each formed therein and having a through hole (601, 602, 603, 604), a first opening (611-1, 612-1, 613-1, 614-1) formed at the top, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member (620b) having a plurality of second regions each formed therein and having a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured into the first opening and holding or retaining

a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, a third member (630) having a plurality of third regions each formed therein and having a hydrophobic third filter, a transparent fourth member (620a) having a plurality of fourth regions each formed therein and having a through hole (611'-2, 612'-2, 613'-2, 614'-2) and a second opening (611-2, 612-2, 613-2, 614-2) at the top, and a means (640) for controlling the temperature of the PCR amplification reaction mixture, the first member, the fourth member, the second member and the third member being arranged in that order from above, the first through hole and the second filter being opposed to each other via the second through hole, the second filter and the third filter being opposed to each other, and a channel being formed for connecting the first filter to the second filter; an irradiation means for irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam (380) capable of exciting the fluorophore label labeling the copy in the PCR reaction mixture in the direction substantially parallel to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units; and a detection means for detecting, substantially

simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter.

35. A method of gene examination using a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second and a third region, and thus comprising a first member (500, 600) having a plurality of first regions each formed therein and having a first opening (511-1, 512-1, 513-1, 514-1; 611-1, 612-1, 613-1, 614-1) formed at the top and a first filter formed in the lower part thereof, a second member (520, 620) having a plurality of second regions each formed therein and having a second opening (511-2, 512-2, 513-2, 514-2; 611-2, 612-2, 613-2, 614-2; 611'-2, 612'-2, 613'-2, 614'-2) formed at the top and a second filter in the lower part thereof and a third member (530, 630) having a plurality of third regions each formed therein and having a hydrophobic third filter, with the first member, the second member and the third member being arranged in that order from above, which method comprises (1) the step of pouring a buffer solution with cells suspended therein into the first opening and capturing the cells on the first filter, (2) the step of pouring a denaturing agent into the first opening and capturing polynucleotides thereby

eluted from the cells on the second filter, (3) pouring a PCT amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, into the first opening, allowing the PCR amplification reaction mixture to be held or retained by the second filter and amplifying the copy, (4) the step of irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam 380 capable of exciting the fluorophore label labeling the copy in the direction substantially perpendicular to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units and (5) the step of detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter.

36. A method of gene examination using a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member (500) having a plurality of first regions each formed therein and having a first opening (511-1,

512-1, 513-1, 514-1) formed at the top and a first filter formed in the lower part thereof, a second member (520) having a plurality of second regions each formed therein and having a second opening (511-2, 512-2, 513-2, 514-2) at the top and a second filter in the lower part thereof, a third member (530) having a plurality of third regions each formed therein and having a hydrophobic third filter and a transparent fourth member (510) having a plurality of fourth regions each formed therein and having a through hole (511-5, 512-5, 513-5, 514-5), with the first member, the fourth member, the second member and the third member being arranged in that order from above, the first filter and the second filter being opposed to each other via the through hole and the second filter and the third filter being opposed to each other, which method comprises (1) the step of pouring a buffer solution with cells suspended therein into the first opening and capturing the cells on the first filter, (2) the step of pouring a denaturing agent into the first opening and capturing polynucleotides thereby eluted from the cells on the second filter, (3) pouring a PCT amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, into the first opening, allowing the PCR amplification reaction

mixture to be held or retained and amplifying the copy, (4) the step of irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam 380 capable of exciting the fluorophore label labeling the copy in the PCR amplification mixture in each sample preparation unit in the direction substantially parallel to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units and (5) the step of detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter in each sample preparation unit.

37. A method of gene examination using a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second and a third region, and thus comprising a first member (600) having a plurality of first regions each formed therein and having a first through hole (601, 602, 603, 604), a first opening (611-1, 612-1, 613-1, 614-1) formed at the top and a first filter formed below the first opening, a second member (620) having a plurality of second regions each formed therein and having a second opening (611-2, 612-2, 613-2, 614-2; 611'-2, 612'-2, 613'-2, 614'-2) at the top and a second filter

in the lower part thereof and a third member (630) having a plurality of third regions each formed therein and having a hydrophobic third filter, with the first member, the second member and the third member being arranged in that order from above, the first through hole and the second opening (611'-2, 612'-2, 613'-2, 614'-2) being opposed to each other, the second filter and the third filter being opposed to each other and a channel being formed for connecting the first filter to the second filter, which method comprises (1) the step of pouring a buffer solution with cells suspended therein into the first opening and capturing the cells on the first filter, (2) the step of pouring a denaturing agent into the first opening and capturing polynucleotides thereby eluted from the cells on the second filter, (3) pouring a PCT amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, into the first opening, allowing the PCR amplification reaction mixture to be held or retained and amplifying the copy, (4) the step of irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam (380) capable of exciting the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially

perpendicular to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units and (5) the step of detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter in each sample preparation unit.

38. A method of gene examination using a sample preparation instrument comprising a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member (600) having a plurality of first regions each formed therein and having a first through hole (601, 602, 603, 604), a first opening (611-1, 612-1, 613-1, 614-1) formed at the top, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member (620b) having a plurality of second regions each formed therein and having a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured from the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence

in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member (630) having a plurality of third regions each formed therein and having a hydrophobic third filter and a transparent fourth member (620a) having a plurality of fourth regions each formed therein and having a second through hole (611'-2, 612'-2, 613'-2, 614'-2) and a second opening (611-2, 612-2, 613-2, 614-2) at the top, with the first member, the fourth member the second member and the third member being arranged in that order from above, the first through hole and the second filter being opposed to each other via the second through hole, the second filter and the third filter being opposed to each other and a channel being formed for connecting the first filter to the second filter, which method comprises (1) the step of pouring a buffer solution with cells suspended therein into the first opening and capturing the cells on the first filter, (2) the step of pouring a denaturing agent into the first opening and capturing polynucleotides thereby eluted from the cells on the second filter, (3) pouring a PCT amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore, into the first opening, allowing the PCR amplification reaction mixture to be held or retained and amplifying the copy, (4) the step

of irradiating the PCR amplification reaction mixture in each sample preparation unit with a laser beam (380) capable of exciting the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially parallel to the second filter in each sample preparation unit substantially simultaneously for the plurality of sample preparation units and (5) the step of detecting, substantially simultaneously for the plurality of sample preparation units, the fluorescence from the fluorophore label labeling the copy in the PCR amplification reaction mixture in each sample preparation unit in the direction substantially perpendicular to the second filter.

39. A sample preparation instrument which has a plurality of sample preparation units, each having a first, a second and a third region, and thus comprising a first member (500, 600) having a plurality of first regions each formed therein and having a first opening (511-1, 512-1, 513-1, 514-1; 611-1, 612-1, 613-1, 614-1) formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed in the lower part thereof for capturing the cells, a second member (520, 620) having a plurality of second regions each formed therein and having a second opening (511-2, 512-2, 513-2, 514-2; 611-2, 612-2, 613-2, 614-2; 611'-2, 612'-2, 613'-2, 614'-2) formed at the top thereof and a second filter,

in the lower part thereof, for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member (530, 630) having a plurality of third regions each formed therein and having a hydrophobic third filter, and a means (540, 640) for controlling the temperature of the PCR amplification reaction mixture, the first member, the second member and the third member being arranged in that order from above.

40. A sample preparation instrument which comprises a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member (500) having a plurality of first regions each formed therein and having a first opening (511-1, 512-1, 513-1, 514-1) formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed in the lower part thereof for capturing the cells, a second member (520) having a plurality of second regions each formed therein and having a second opening (511-2, 512-2, 513-2, 514-2) at the top thereof and a second filter, in the lower part thereof, for capturing polynucleotides eluted from the cells by

means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member (530) having a plurality of third regions each formed therein and having a hydrophobic third filter, a transparent fourth member (510) having a plurality of fourth regions each formed therein and having a through hole (511-5, 512-5, 513-5, 514-5), and a means (540) for controlling the temperature of the PCR amplification reaction mixture, the first member, the fourth member, the second member and the third member being arranged in that order from above, the first filter and the second filter being opposed to each other via the through hole, and the second filter and the third filter being opposed to each other.

41. A sample preparation instrument which comprises a plurality of sample preparation units, each having a first, a second and a third region, and thus comprising a first member (600) having a plurality of first regions each formed therein and having a first through hole (601, 602, 603, 604), a first opening (611-1, 612-1, 613-1, 614-1) formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member

(620) having a plurality of second regions each formed therein and having a second opening (611-2, 612-2, 613-2, 614-2; 611'-2, 612'-2, 613'-2, 614'-2) at the top and a second filter, in the lower part thereof, for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member (630) having a plurality of third regions each formed therein and having a hydrophobic third filter, and a means (640) for controlling the temperature of the PCR amplification reaction mixture, the first member, the second member and the third member being arranged in that order from above, the first through hole and the second opening (611'-2, 612'-2, 613'-2, 614'-2) being opposed to each other, the second filter and the third filter being opposed to each other, and a channel being formed for connecting the first filter to the second filter.

42. A sample preparation instrument which comprises a plurality of sample preparation units, each having a first, a second, a third and a fourth region, and thus comprising a first member (600) having a plurality of first regions each formed therein and having a first through hole (601, 602, 603, 604), a

first opening (611-1, 612-1, 613-1, 614-1) formed at the top thereof, into which a buffer solution with cells suspended therein is poured, and a first filter formed below the first opening for capturing the cells, a second member (620b) having a plurality of second regions each formed therein and having a second filter for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, a third member (630) having a plurality of third regions each formed therein and having a hydrophobic third filter, a transparent fourth member (620a) having a plurality of fourth regions each formed therein and having a second through hole (611'-2, 612'-2, 613'-2, 614'-2) and a second opening (611-2, 612-2, 613-2, 614-2) at the top, and a means (640) for controlling the temperature of the PCR amplification reaction mixture, the first member, the fourth member, the second member and the third member being arranged in that order from above, the first through hole and the second filter being opposed to each other via the second through hole, the second filter and the third filter being opposed to each other, and a channel being formed for connecting the first filter to the second filter.

43. A sample preparation instrument which comprises a sample preparation unit (100) having an upper opening, into which a buffer solution with cells suspended therein is poured, a lower opening for discharging a waste liquor, a channel connecting the openings, a first filter (110) for capturing the cells, a second filter (130) for capturing polynucleotides eluted from the cells by means of a denaturing agent poured through the first opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial base sequence in the polynucleotides, inclusive of a PCR primer labeled with a fluorophore, and a hydrophobic third filter (140), the filters being arranged in that order in the channel in the direction from the upper opening to the lower opening; a holding member (200) for holding the sample preparation unit; and a means (210) for controlling the temperature of the PCR amplification reaction mixture.

ABSTRACT OF THE DISCLOSURE

The sample preparation instrument according to the invention comprises a sample preparation unit (100) comprising an upper opening, into which a buffer solution with cells suspended therein is poured, a lower opening, through which a waste liquor is discharged, and a channel connecting these openings and provided with a first filter (110) for capturing the cells, a second filter (130) for capturing polynucleotides eluted from the cells by means of a denaturing agent poured into the upper opening and holding or retaining a PCR amplification reaction mixture containing reagents for the PCR amplification reaction for amplifying the copy of a desired partial target base sequence in the polynucleotides captured, inclusive of a PCR primer labeled with a fluorophore and a hydrophobic third filter (140), the three filters being arranged in the channel in that order in the direction from the upper opening to the lower opening, a holding member (200) for holding the sample preparation unit and a means (210) for controlling the temperature of the PCR amplification reaction mixture. It makes it possible to carry out all the steps, from sample pretreatment to PCR amplification and PCR product detection, in a continuous manner, thus prevent contamination between steps and facilitate simultaneous treatment of a number of specimens.

FIG. 1

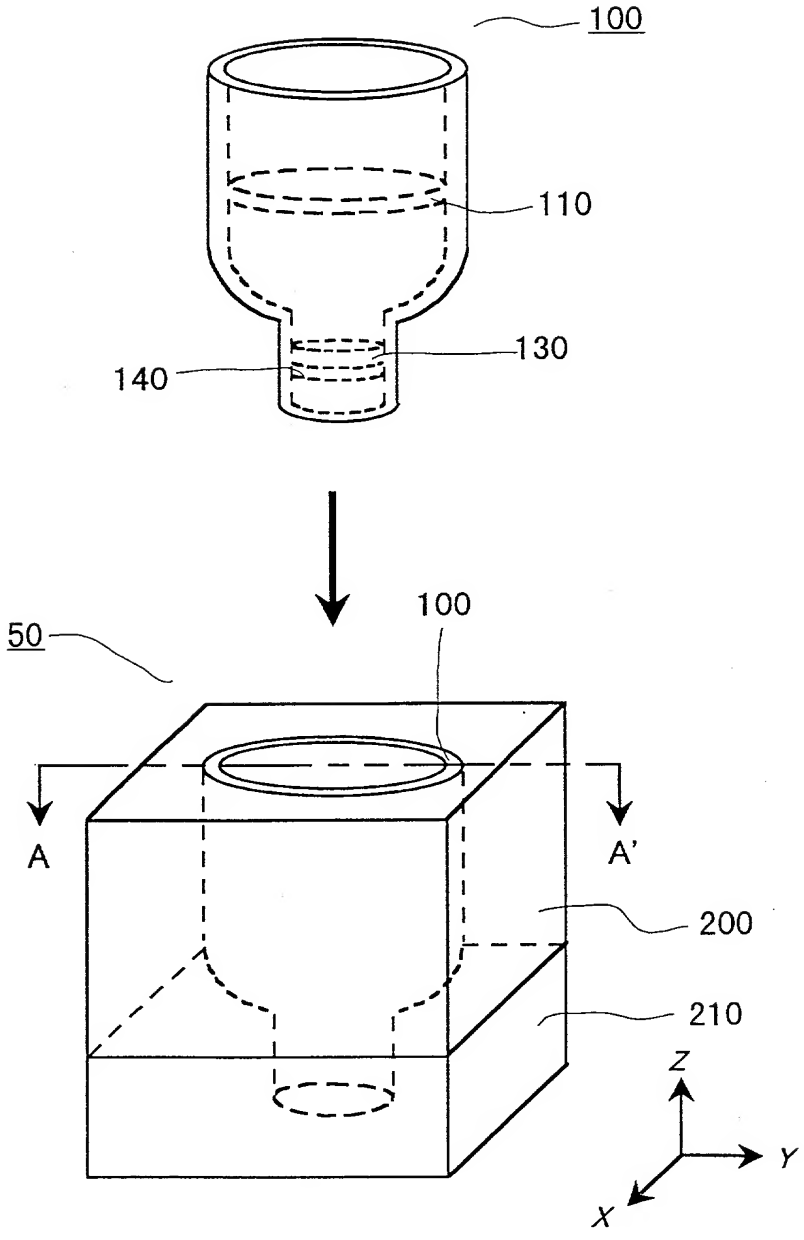


FIG. 2

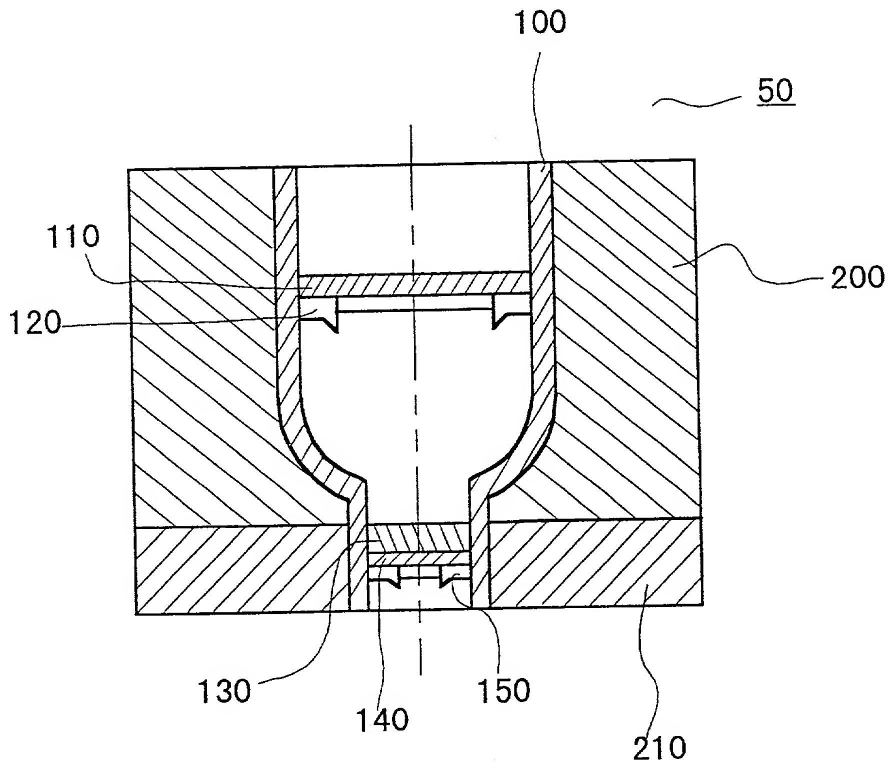
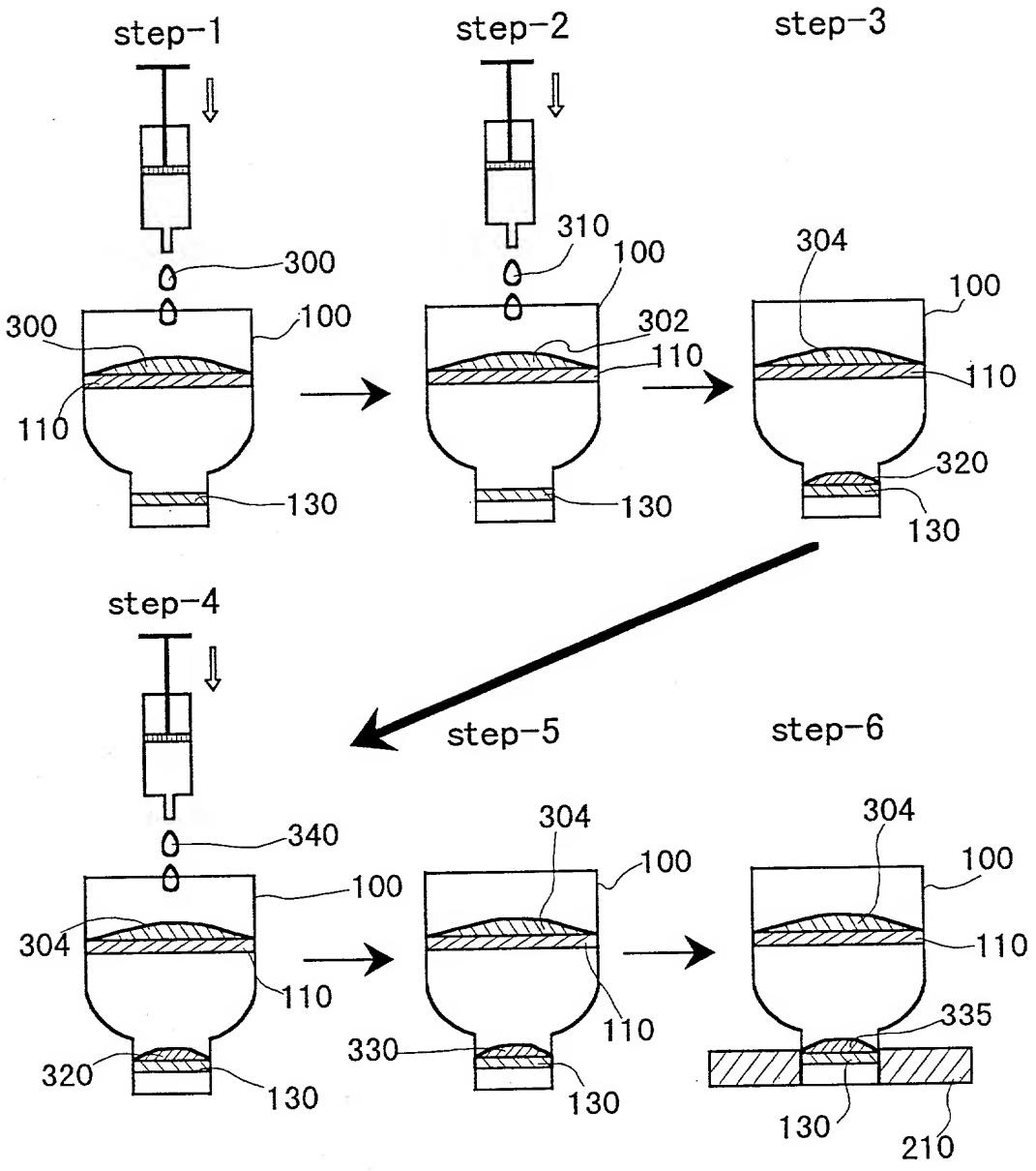


FIG. 3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step-2
step-3
step-4
step-5
step-6
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100
110
130
310
302
304
320
340
330
335
210

FIG. 4

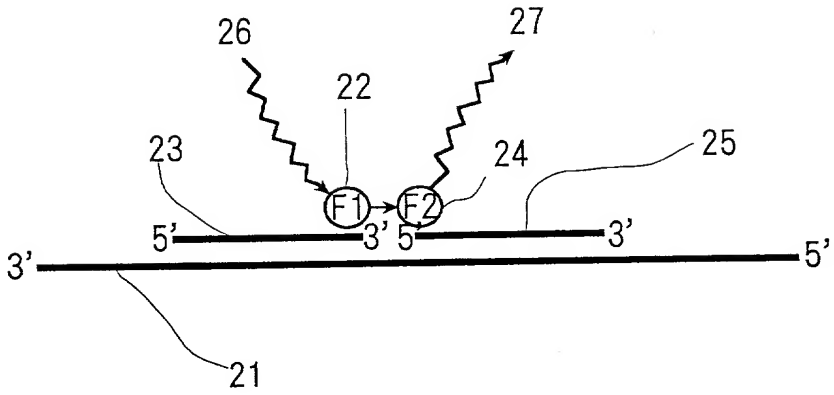
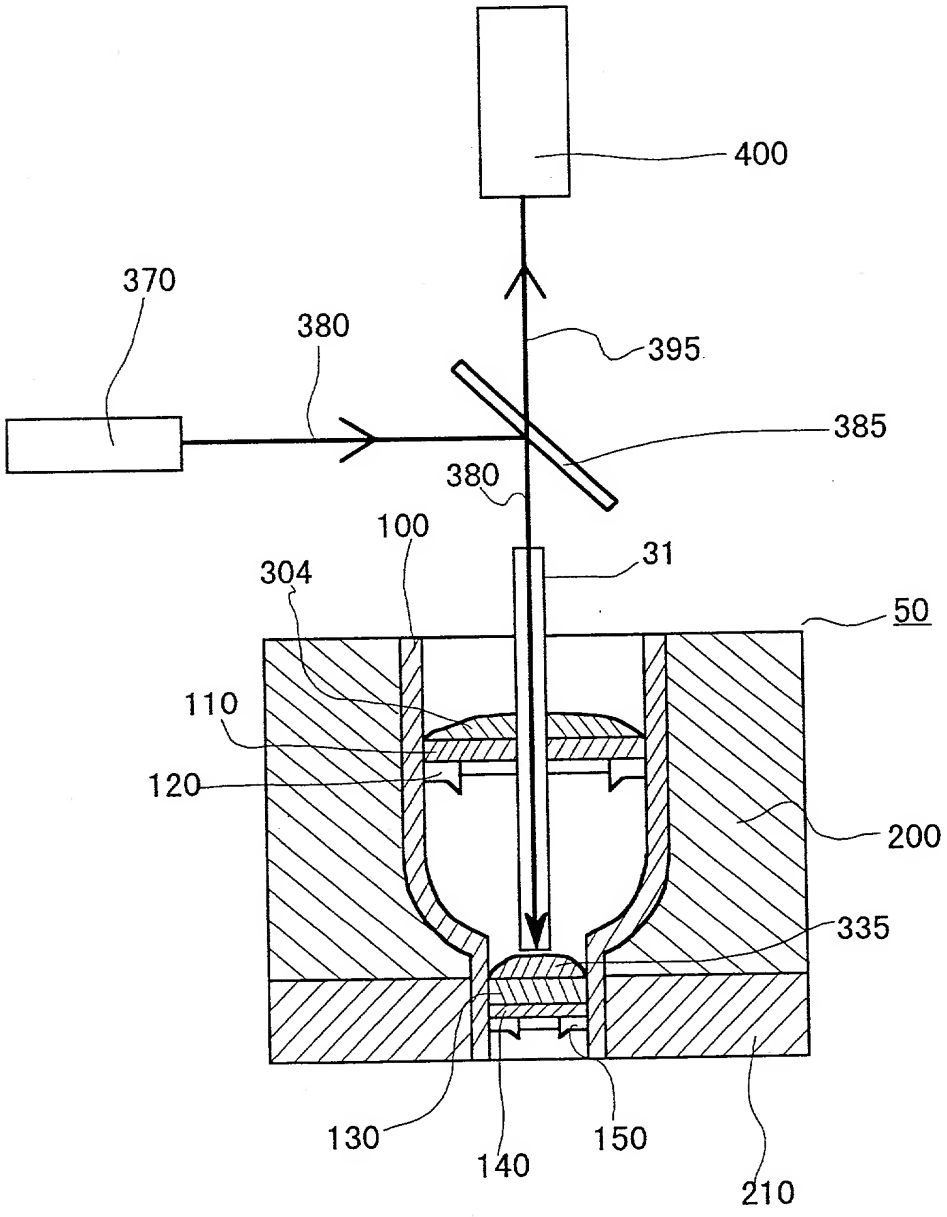


FIG. 5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210

FIG. 6

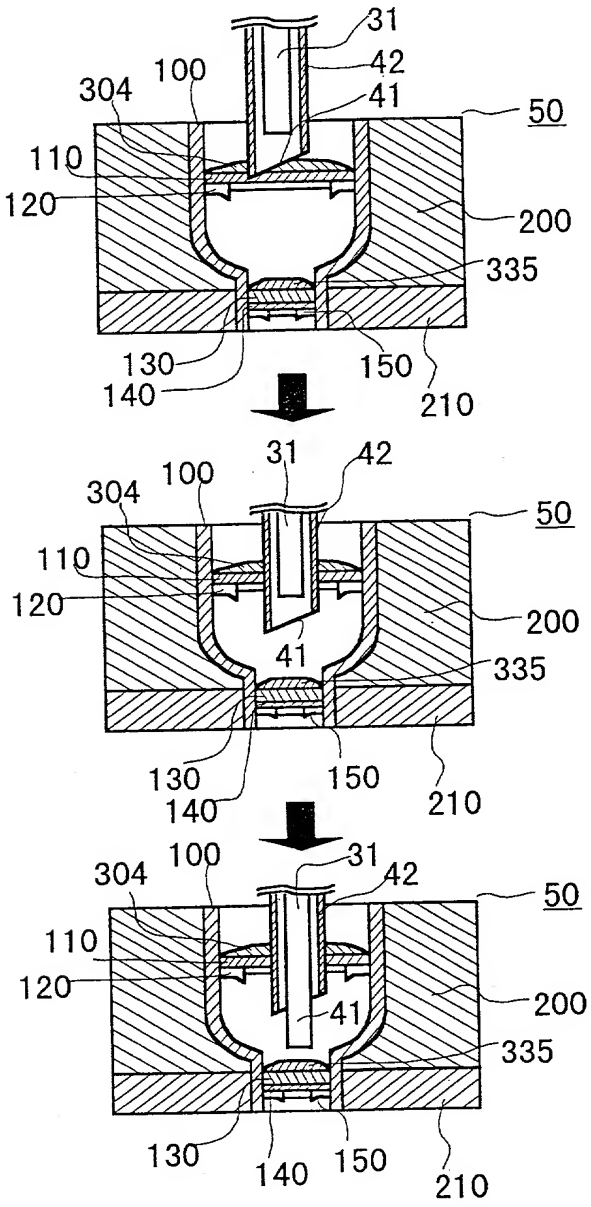
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FIG. 7

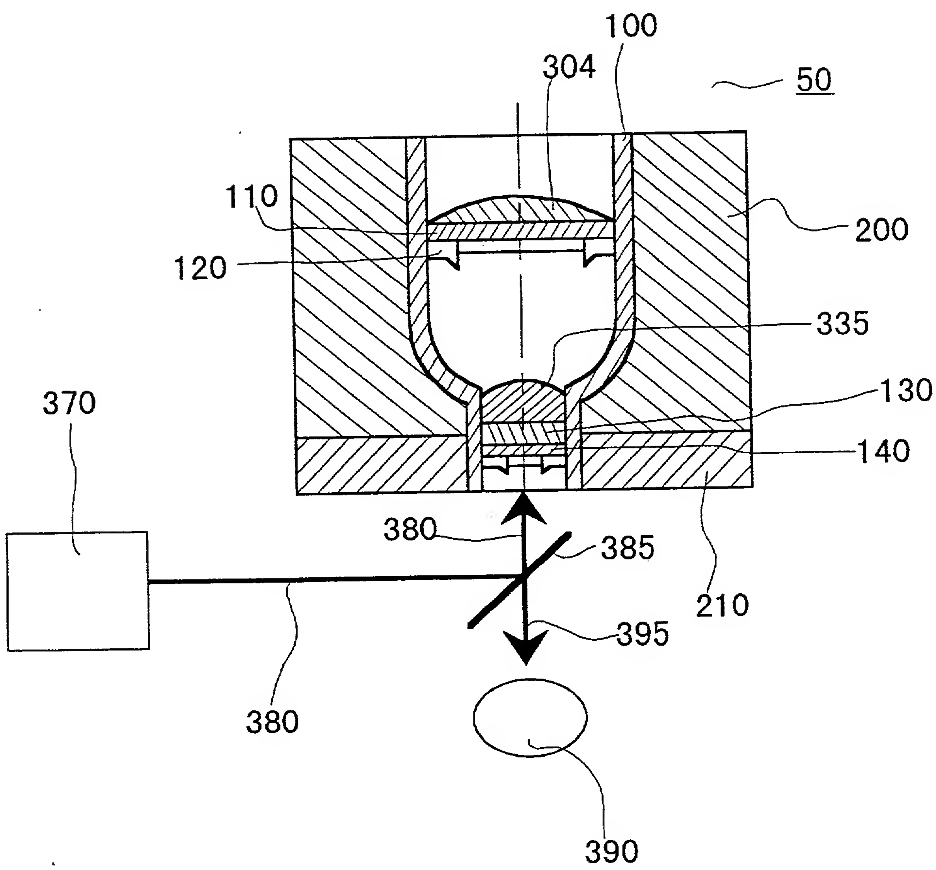


FIG. 8

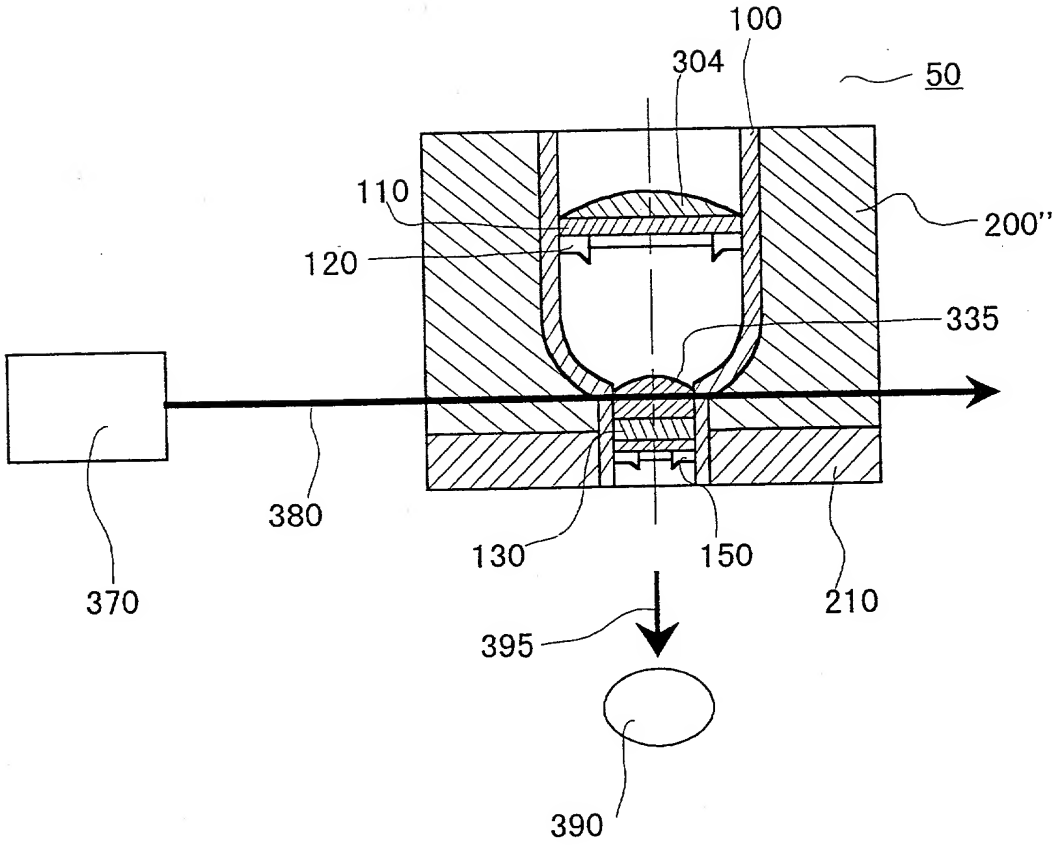


FIG. 9

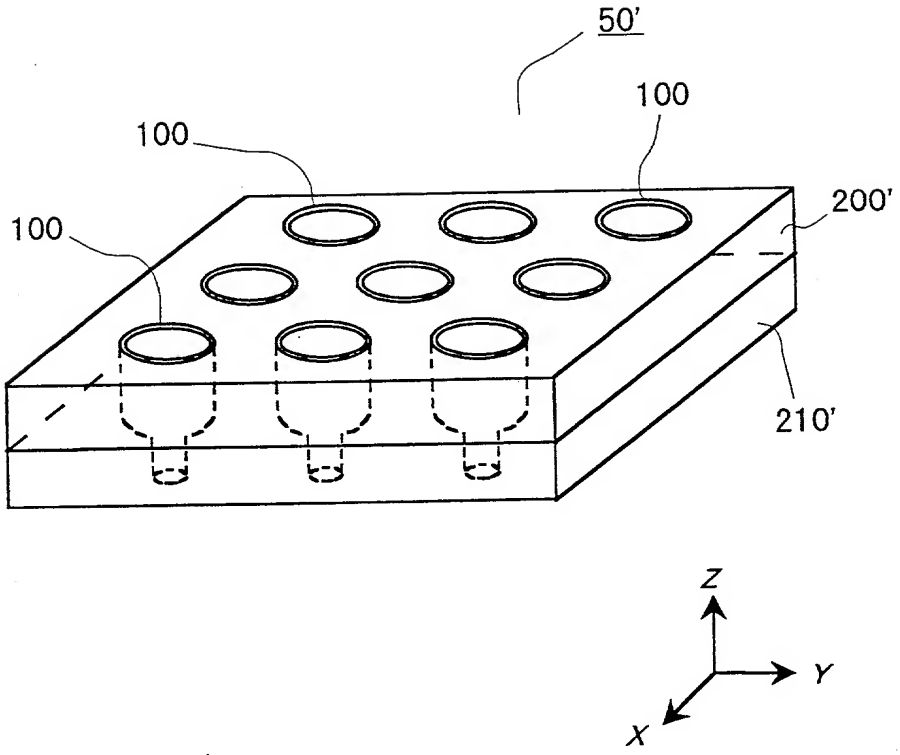
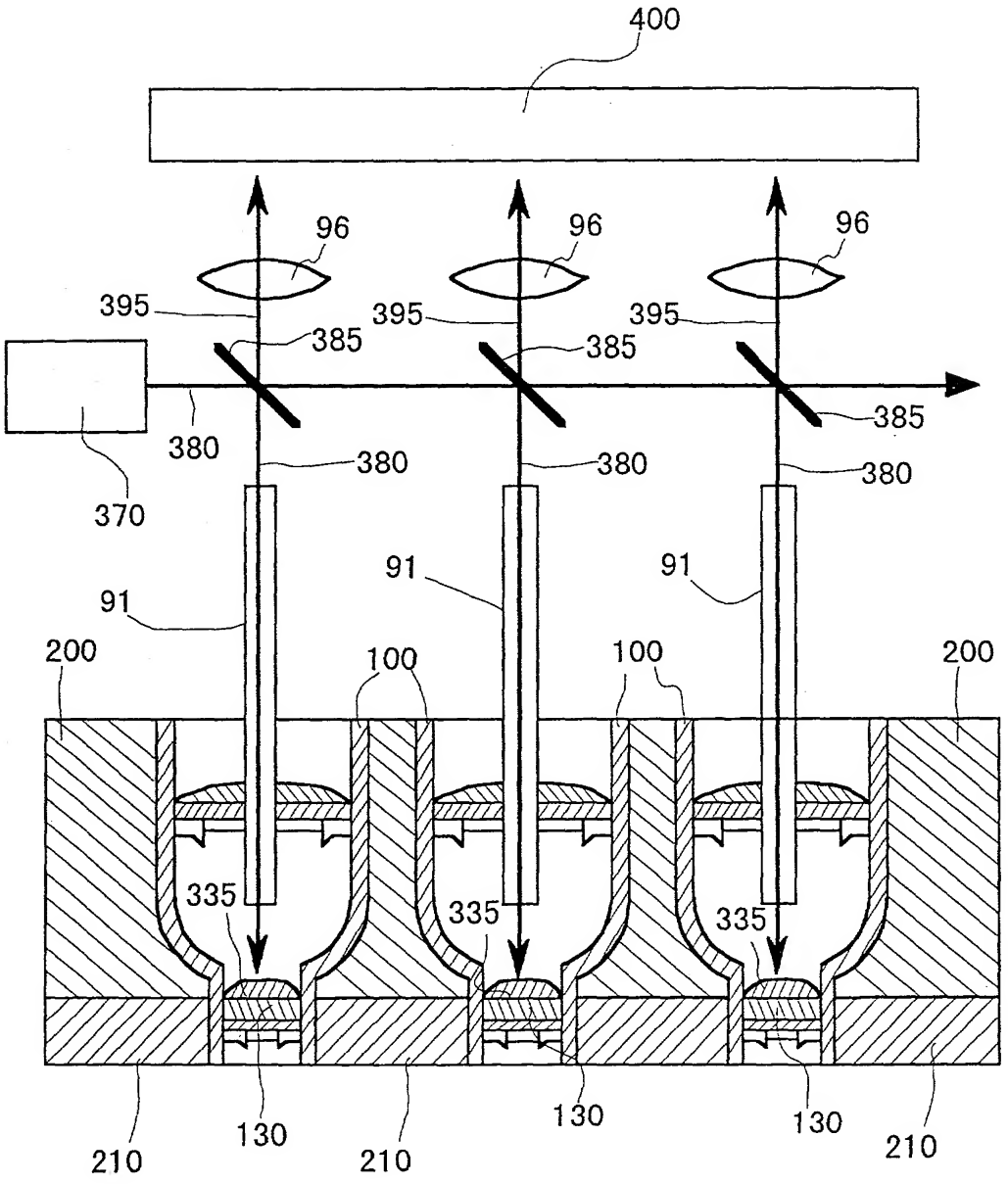


FIG. 10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130
130
130
210
210
210

FIG. 11

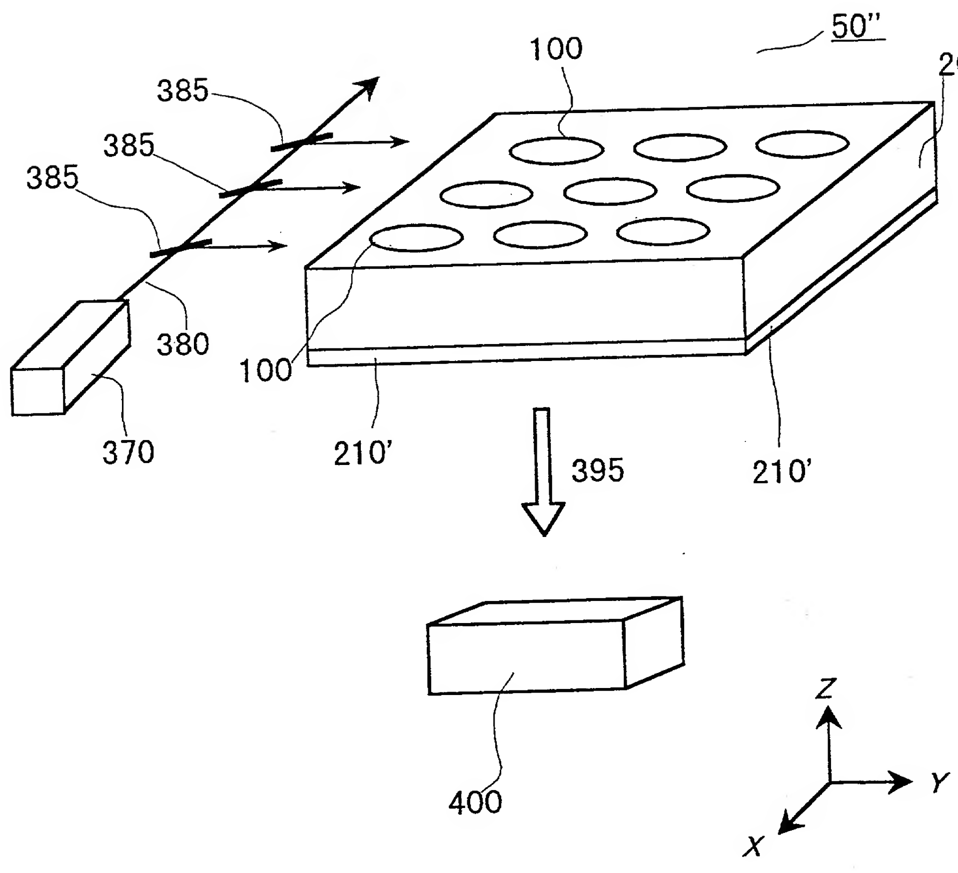

FIG. 12

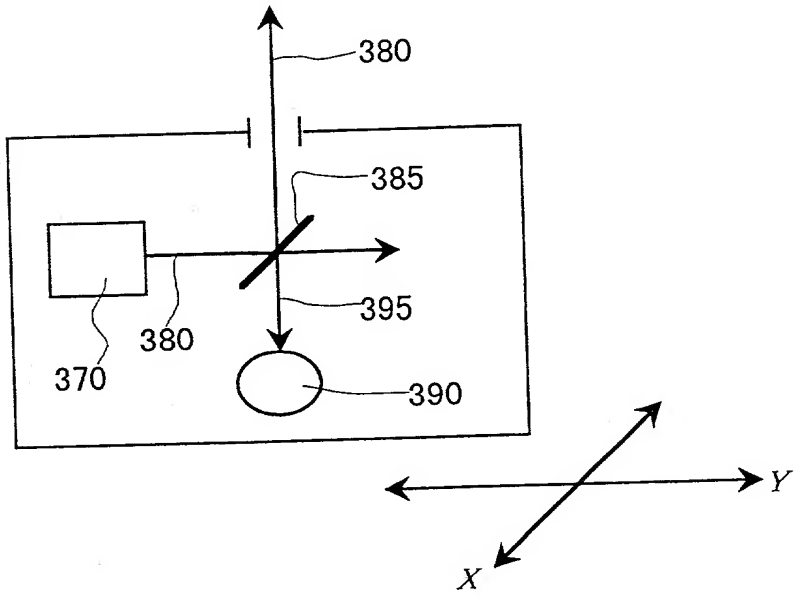

FIG. 13

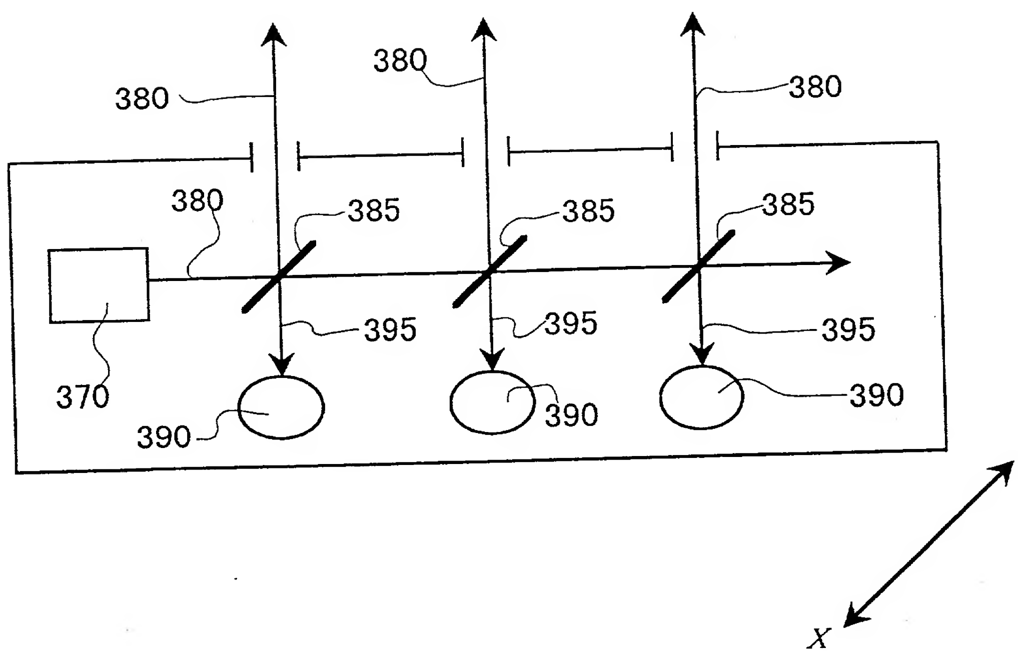

FIG. 14

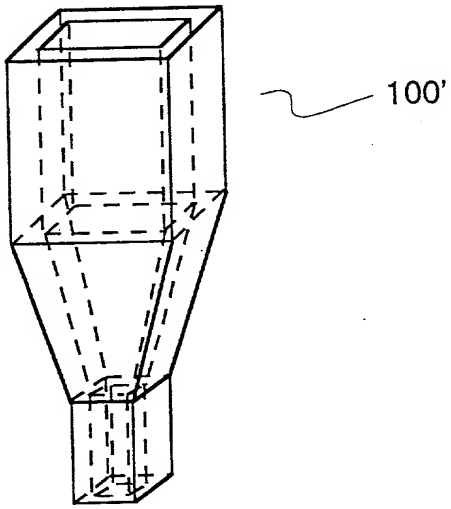

FIG. 15

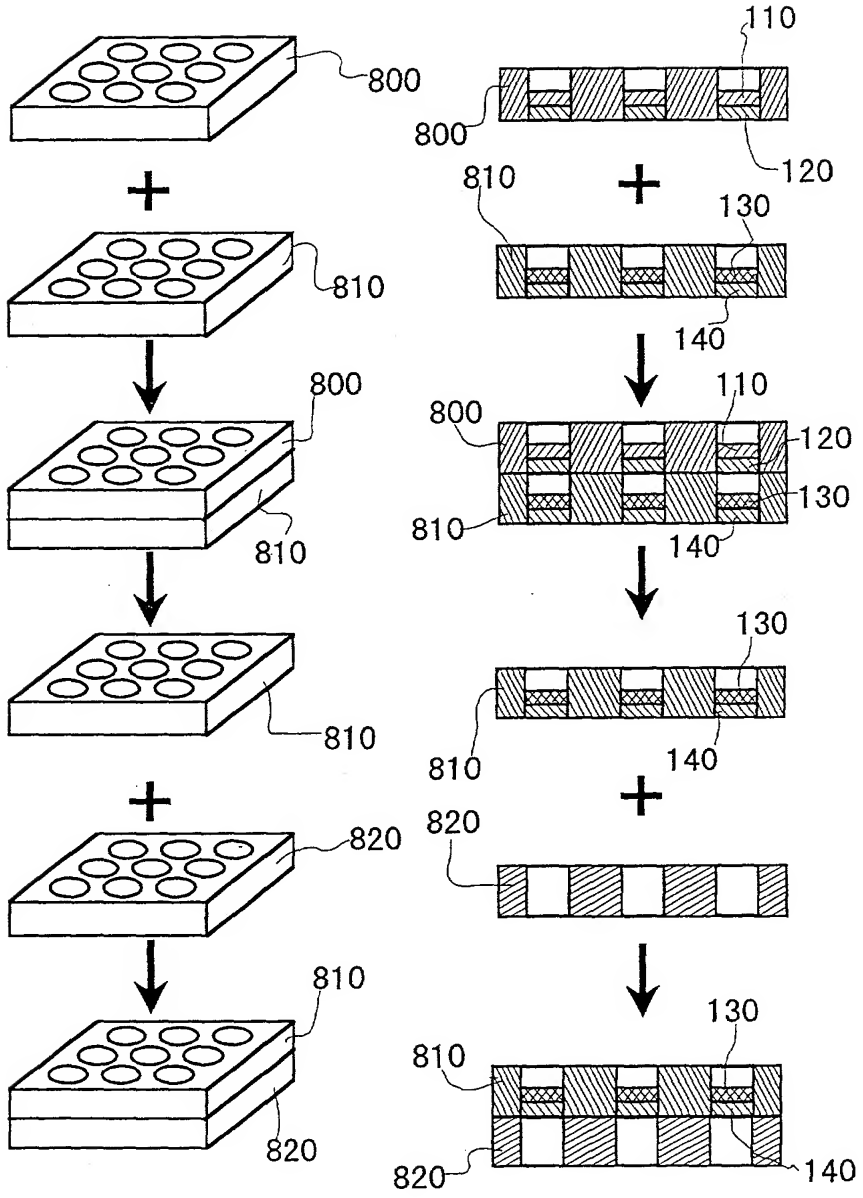

FIG. 16

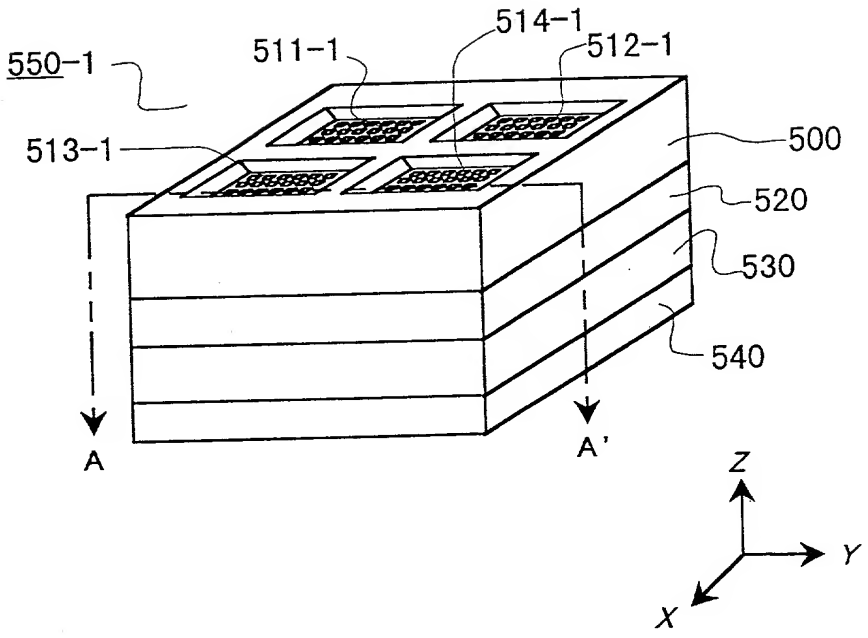

FIG. 17

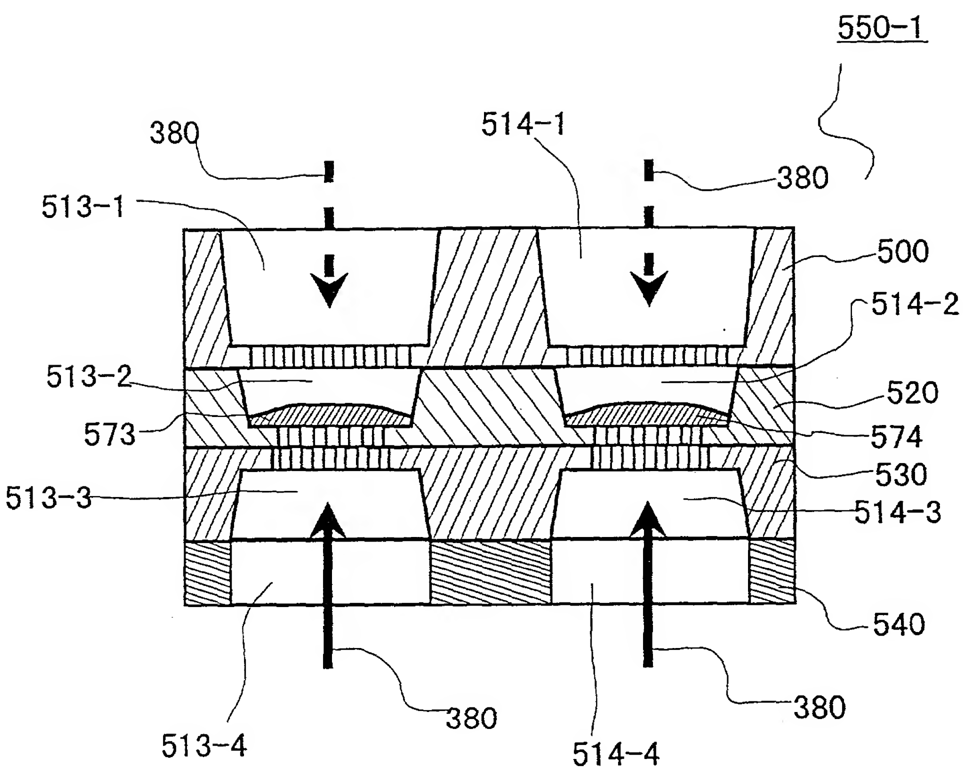

FIG. 18

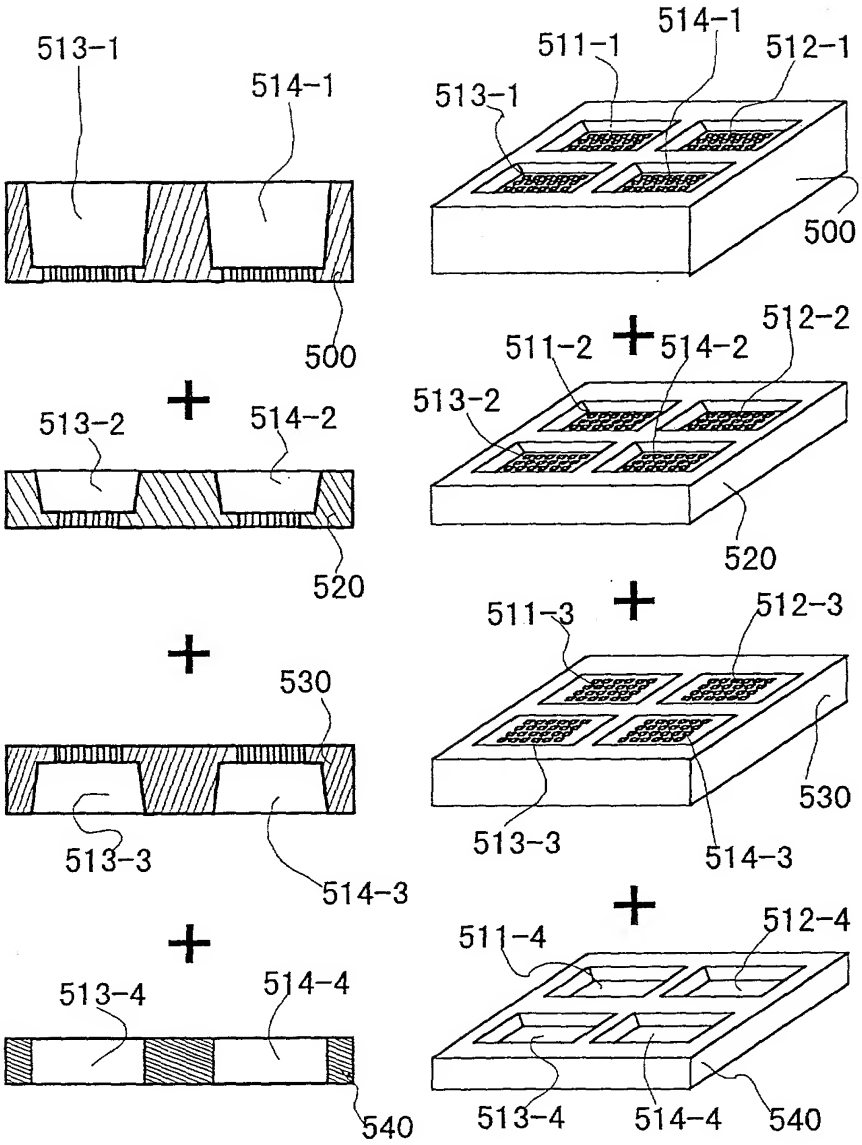
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FIG. 19

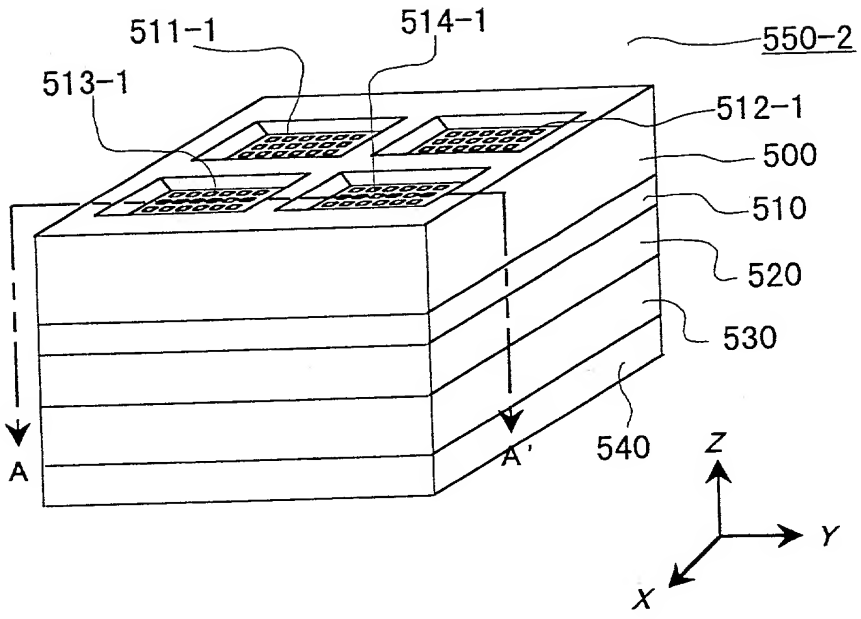

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FIG. 20

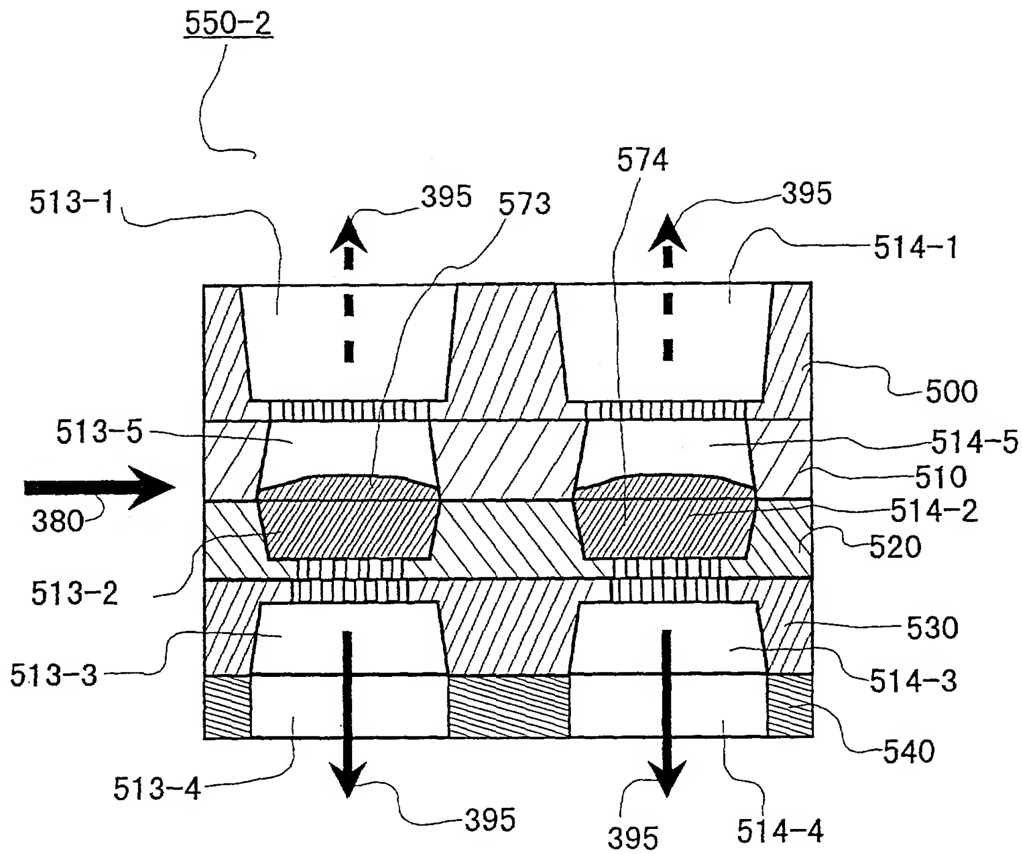

FIG. 21

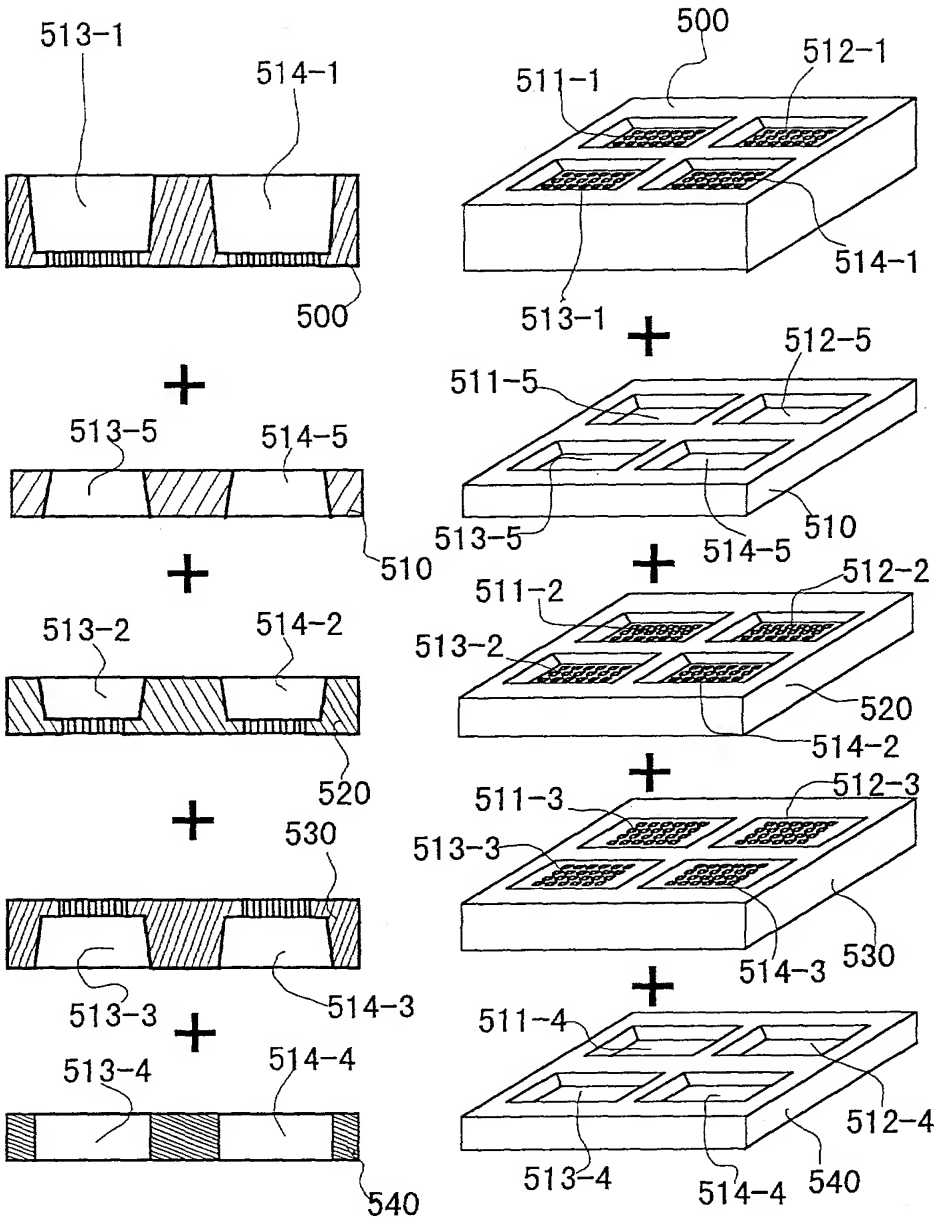
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FIG. 22

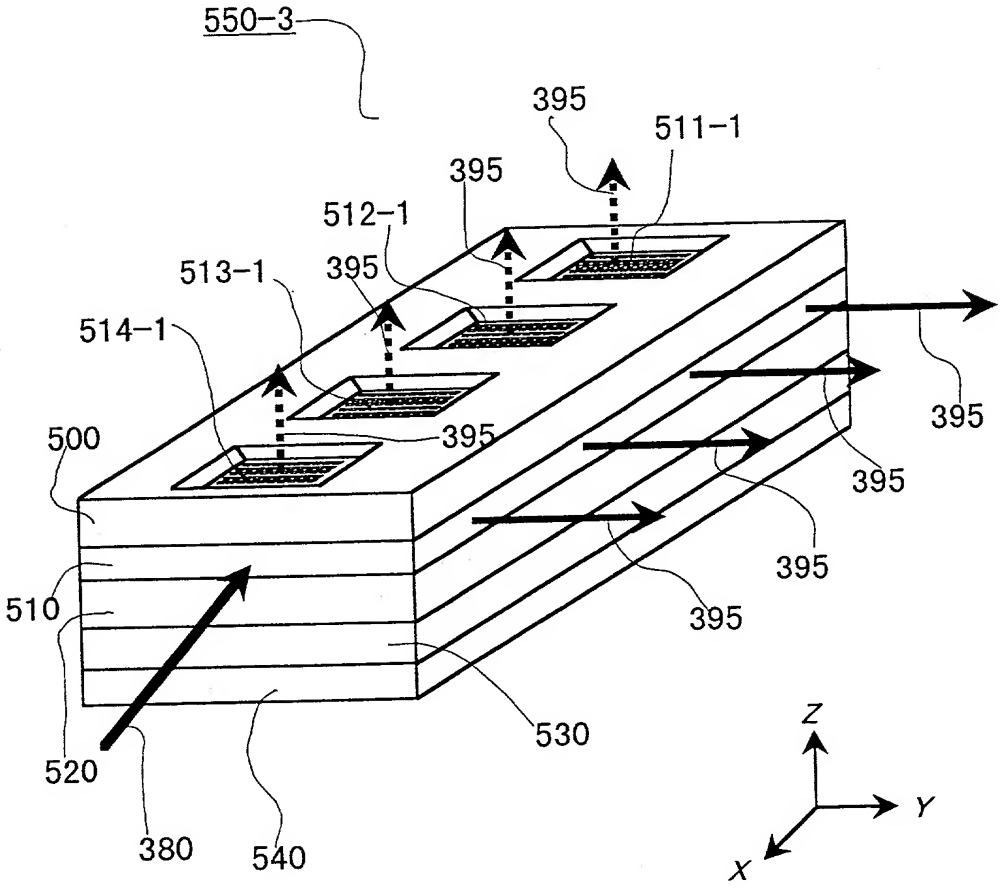
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FIG. 23

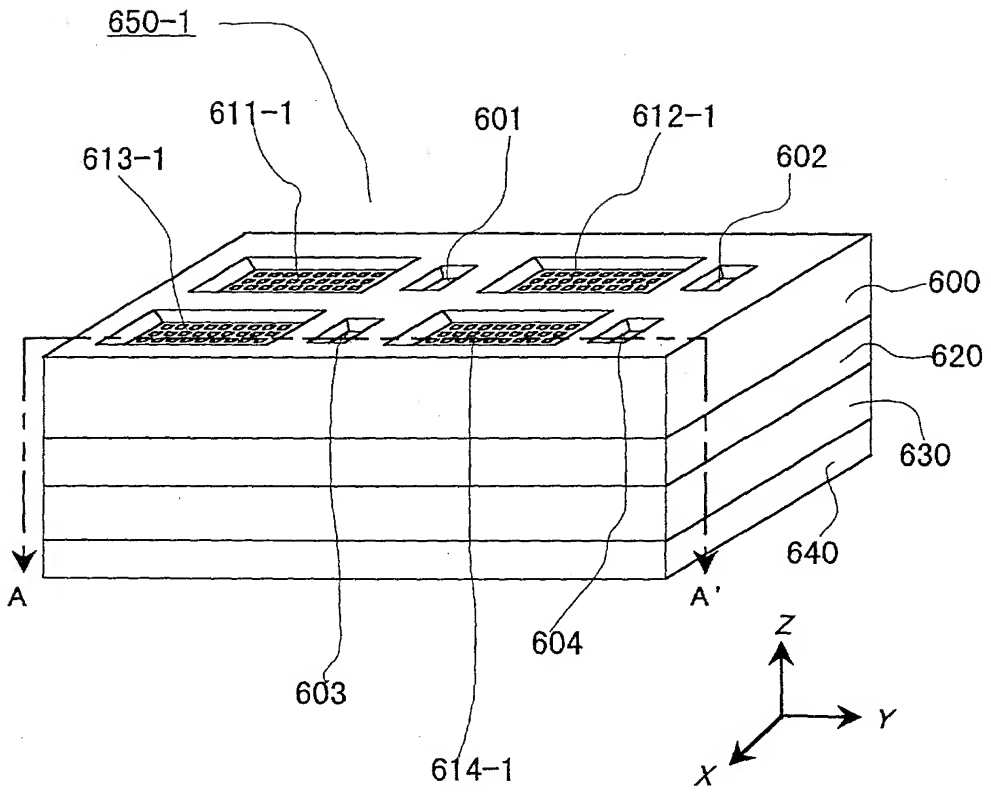

FIG. 24

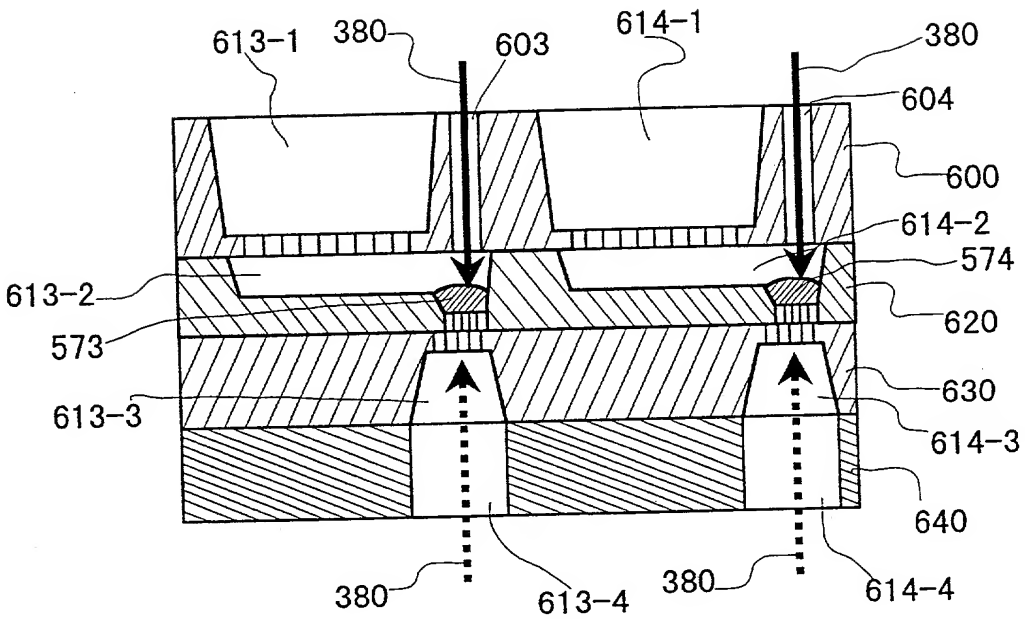
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FIG. 25

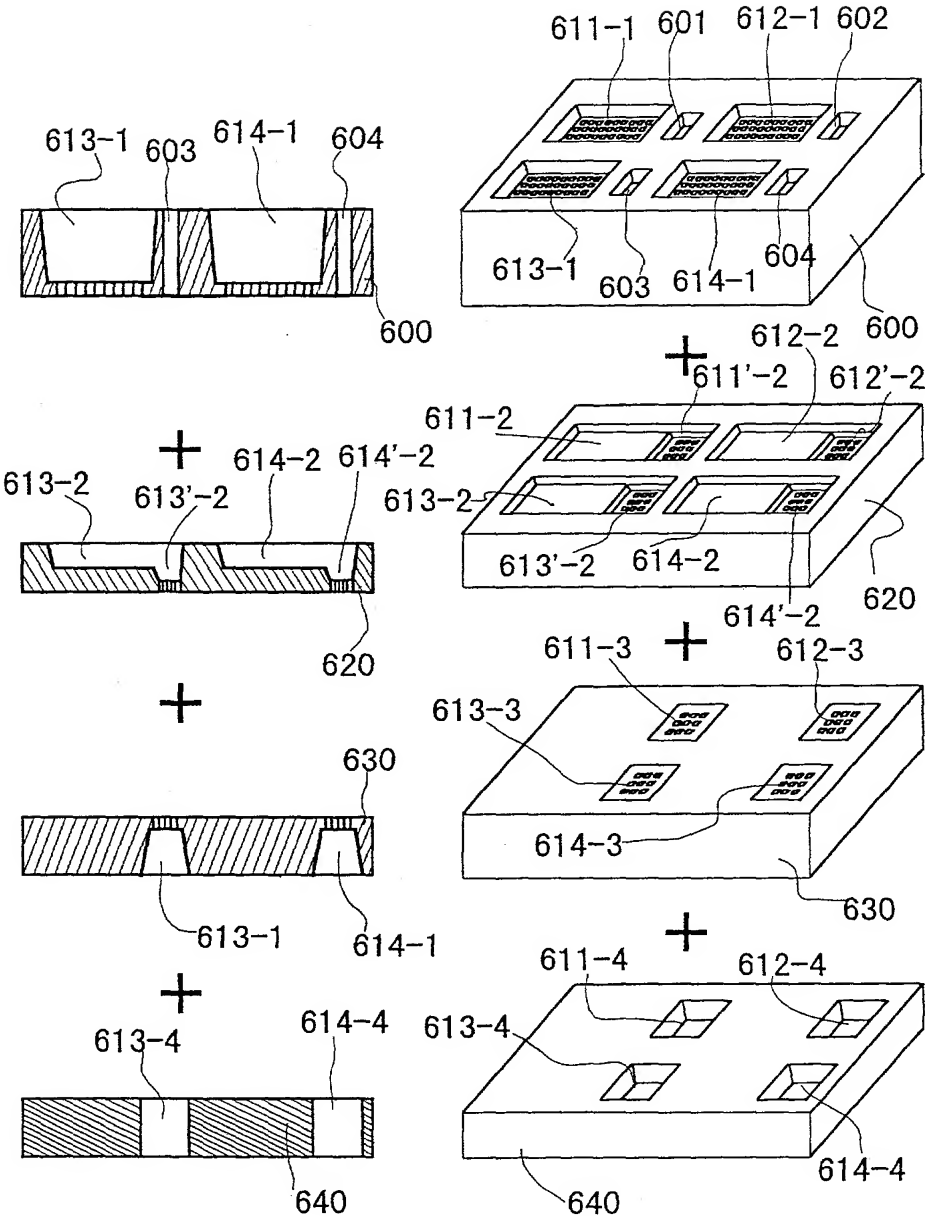
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FIG. 26

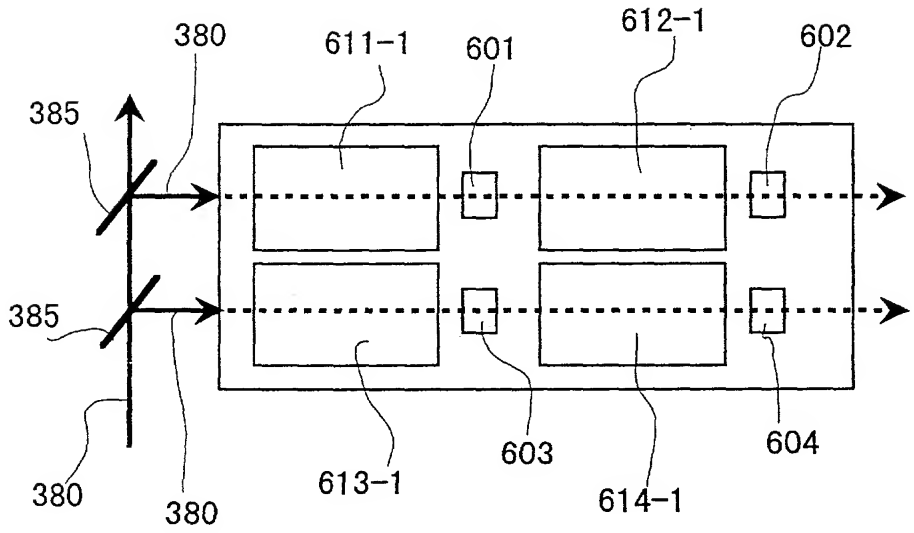
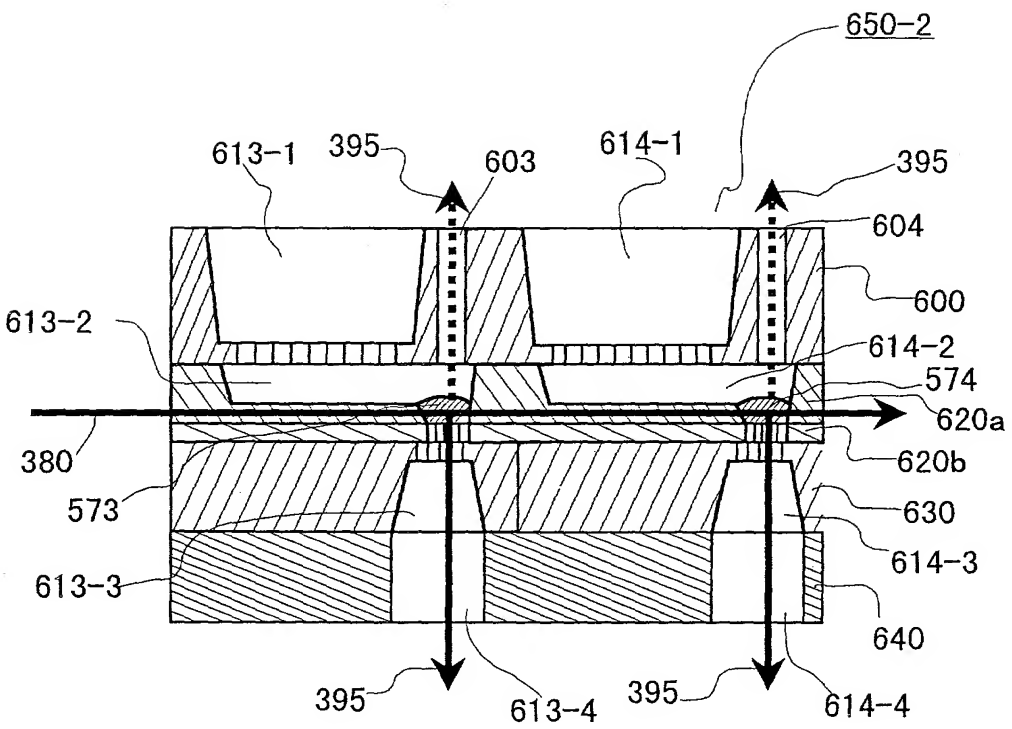


FIG. 27



PTO/SB/106(8-96)
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Patent and Trademark Office; U.S. DEPARTMETNT OF COMMERCE

Declaration and Power of Attorney For Patent Application

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

As a below named inventor, I hereby declare that:

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

My residence, post office address and citizenship are as stated next to my name.

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者であると（下記の名称が複数の場合）信じています。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

APPARATUS AND METHOD FOR GENE EXAMINATION

上記発明の明細書（下記の欄で×印がついていない場合は、本書に添付）は、

The specification of which is attached hereto unless the following box is checked:

☐ __月__日に提出され、米国出願番号または特許協定条約国際出願番号を_____とし、
(該当する場合) _____に訂正されました。

☒ was filed on 16／June／1999
as United States Application Number or
PCT International Application Number
PCT／JP99／03209 and was amended on
_____ (if applicable).

私は、特許請求範囲を含む上記訂正後の明細書を検討し、内容を理解していることをここに表明します。

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第３７編第１条５６項に定義されるとおり、特許資格の有無について重要な情報を開示する義務があることを認めます。

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

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Japanese Language Declaration
(日本語宣言書)

私は、米国法典第３５編１１９条 (a) - (d) 項又は３６５条 (b) 項に基き下記の、米国以外の国の少なくとも一カ国を指定している特許協力条約３６５ (a) 項に基ずく国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している、本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示している。

I hereby claim foreign priority under Title 35, United States Code, Section 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)
外国での先行出願

Priority Not Claimed
優先権主張なし

(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願年月日)	☐
(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願年月日)	☐

私は、第３５編米国法典１１９条 (e) 項に基いて下記の米国特許出願規定に記載された権利をここに主張いたします。

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Filing Date) (出願日)	(Application No.) (出願番号)	(Filing Date) (出願日)

私は、下記の米国法典第３５編１２０条に基いて下記の米国特許出願に記載された権利、又は米国を指定している特許協力条約３６５条 (c) に基ずく権利をここに主張します。また、本出願の各請求範囲の内容が米国法典第３５編１１２条第１項又は特許協力条約で規定された方法で先行する米国特許出願に開示されていない限り、その先行米国出願書提出日以降で本出願書の日本国内または特許協力条約国際提出日までの期間中に入手された、連邦規則法典第３７編１条５６項で定義された特許資格の有無に関する重要な情報について開示義務があることを認識しています。

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s), or 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of application.

(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況：特許許可済、係属中、放棄済)
(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況：特許許可済、係属中、放棄済)

私は、私自身の知識に基ずいて本宣言書中で私が行なう表明が真実であり、かつ私の入手した情報と私の信じるところに基ずく表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第１８編第１００１条に基ずき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行なえば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Patent and Trademark Office; U.S. DEPARTMETNT OF COMMERCE

Japanese Language Declaration
(日本語宣言書)

委任状： 私は下記の発明者として、本出願に関する一切の手続きを米特許商標局に対して遂行する弁理士または代理人として、下記の者を指名いたします。（弁護士、または代理人の氏名及び登録番号を明記のこと）

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith (list name and registration number)

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Direct Telephone Calls to: (*name and telephone number*)

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発明者の署名 日付	Inventor's signature Date Hiroko Matsunaga 11/12/2001
住所	Residence ~~Ames, IOWA~~ FA
国籍	Citizenship Japan
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(第二以降の共同発明者についても同様に記載し、署名をすること)

(Supply similar information and signature for second and subsequent joint inventors.)

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第二共同発明者の署名	日付	Second inventor's signature Date Katsuji Murakawa 11/30/2001
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国籍		Citizenship Japan
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第三共同発明者の署名	日付	Third inventor's signature Date Kazunori Okano 12/5/2001
住所		Residence Shiki, Japan JPX
国籍		Citizenship Japan
私書箱		Post Office Address c/o Hitachi, Ltd., Intellectual Property Group New Marunouchi Bldg. 5-1, Marunouchi 1-chome, Chiyoda-ku, Tokyo 100-8220, Japan
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第四共同発明者の署名	日付	Fourth inventor's signature Date Yuji Miyahara 12/10/2001
住所		Residence Kodaira, Japan JPX
国籍		Citizenship Japan
私書箱		Post Office Address c/o Hitachi, Ltd., Intellectual Property Group New Marunouchi Bldg. 5-1, Marunouchi 1-chome, Chiyoda-ku, Tokyo 100-8220, Japan
第五共同発明者		Full name of fifth joint inventor, if any
第五共同発明者の署名	日付	Fifth inventor's signature Date
住所		Residence
国籍		Citizenship
私書箱		Post Office Address

(第六以降の共同発明者についても同様に記載し、署名をすること)

(Supply similar information and signature for sixth and subsequent joint inventors.)

PCT
GENERAL POWER OF ATTORNEY
(for several international applications filed under the Patent Cooperation Treaty)

(PCT Rule 90.5)

The undersigned applicant:

DYNAPEL SYSTEMS, INC.

hereby appoints as agents:

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Richard L. AITKEN
Zayd ALATHARI
Ralph P. ALBRECHT
Robert S. BABAYI
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Fei-Fei CHAO
Robert J. FRANK
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Michael A. SARTORI
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Gary L. SHAFFER
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VENABLE
P.O. Box 34385
Washington, DC 20043-9998

to act on applicant's behalf before the competent International Authorities in connection with any and all international applications filed by the applicant with the United States Patent and Trademark Office as receiving Office and to make and receive payments on applicant's behalf.

Signature: S D Edelson
Stephen D. Edelson
Title: Chief Executive Officer

Date: Oct 31/01

ATTORNEY DOCKET NO. 36719 – (300670V1)